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DEVELOPMENT AND VALIDATION OF METHODS FOR APPLYING PHARMACOKINETIC DATA IN RISK ASSESSMENT

VOLUME IV OF VII: METHYLCHLOROFORM

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

JAMES N. McDOUGAL, Maj, USAF, BSC
Deputy Director, Toxic Hazards Division
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FOREWORD

This report has been prepared by Clement International Corporation, K.S. Crump Division, for the Department of the Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory, Wright Paterson Air Force Base in response to a request to investigate the incorporation of pharmacokinetic modeling into quantitative risk assessment. This report contains the results of this multiyear effort and reflects the changes in direction and priorities as this project has evolved. The Project Director was Dr. Kenny Crump and the Principal Investigator for this project was Mr. Bruce Allen; other investigators who provided technical support and internal peer review were Drs. Crump and Annette Shipp. Mr. Allen was assisted in the pharmacokinetic modeling and analyses primarily by Mr. Christopher Rambin and by Ms. Robinan Gentry. The sensitivity analyses were conducted by Mr. David Farrar, Dr. Crump, Dr. Richard Howe, and Mr. Allen. The software was developed by Ms. Cynthia Van Landingham, Mr. William Fuller, Mr. Eric Brooks, Dr. Howe, and Mr. Allen. The authors wish to acknowledge the support provided by Dr. Jeffery Fisher and Lt. Col. Harvey Clewell, who are at the Harry G. Armstrong Aerospace Medical Research Laboratory, Wright Paterson Air Force Base, and Drs. Melvin Andersen and Michael Gargas, formerly with the Harry G. Armstrong Aerospace Medical Research Laboratory and now with CIIT.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

A. INTRODUCTION

The purpose of this volume is to present the pharmacokinetic modeling and dose-response modeling relevant to the assessment of the risk posed by methyl chloroform (MC) to humans. The emphasis in this document is on one of the major metabolites of MC, trichloroacetic acid (TCA) (EPA, 1984). TCA has been associated with liver tumors in mice when administered directly (Herren-Freund et al., 1987) and has been implicated in the hepatocarcinogenicity of trichloroethylene (TCE) and tetrachloroethylene (PERC). The pharmacokinetic work presented here extends the physiologically based pharmacokinetic (PBPK) models of MC that have been proposed (Reitz et al., 1987, 1988) to include prediction of TCA plasma concentrations and/or TCA urinary excretion. Nolan et al. (1984) have discussed a similar extension; their approach will be compared to the approach presented in this document. Dose metrics based on the extended pharmacokinetic model are defined and used as the basis for dose-response modeling and extrapolation of the results observed in mice to predictions pertinent to humans. Of particular interest in the assessment of MC is the use of other chemicals that are metabolized to TCA (TCE and PERC) to derive risk estimates. The basis for this approach is discussed below. Discussions will be limited to the modeling of mice and humans.

Appendix IV-A presents an overview of the toxicity and pharmacokinetic information for MC. The toxicity information focussed on the liver, the site at which tumors in mice have been observed and the site that is thought to be susceptible to the effects of TCA.

The remainder of this document discusses the steps taken to derive risk estimates for humans exposed to MC. First, the PBPK models that have been

published are discussed. Next is a presentation of the approach used to extend PBPK models of the parent compound, MC, which includes the kinetics of TCA. The predictions of the extended models are compared to the available data that are suitable for quantitative comparisons. Based on such comparisons, one set of parameter values for the mouse and one set of parameter values for humans were selected as the basis for dose metric definition, for use in dose-response modeling, and as the basis for route-to-route and cross-species extrapolation.

For the analysis of the animal data, bioassays of MC, TCE, and PERC were examined. The results of the bioassays were compared on the basis of the TCA-based dose metric: exposures to any of the chemicals that yielded the same value of the dose metric for TCA were assumed to give the same risk. This assumption is evaluated in light of the results observed; i.e., the evidence is examined to determine if risk estimates across chemicals appear to correlate with the values of the TCA-based dose surrogate. Having decided how to use the animal data from the three compounds for dose-response modeling, human liver cancer risk estimates were derived.

B. PBPK MODELS

A PBPK model for MC was proposed by Reitz et al. (1987, 1988). Values of the parameters in Reitz et al. (1987) differed from those in Reitz et al. (1988) (cf. Table IV-1). Bogen and Hall (1989) have worked with the Reitz et al. model, using the parameter estimates presented by Reitz et al. (1987). Appendix IV-B presents the equations in which the parameters displayed in Table IV-1 were used.

Reitz et al. (1987, 1988) determined that their model, in which metabolic parameters for humans were scaled from values obtained in rats, could adequately describe the human MC concentration data reported by Nolan et al. (1984). The Nolan et al. data consisted of MC concentrations in venous blood and expired air during and following a 6-hour inhalation exposure to 35 or 350 ppm.

Similarly, the mouse parameter values given by Reitz et al. (1987, 1988) were scaled from rats. Reitz et al. stated that those parameter values adequately described results obtained by Schumann et al. (1982a). Those results consisted of serial venous blood MC concentration data as well as amounts metabolized, body burdens, and tissue concentrations following 6-hour inhalation exposures to MC at 150 or 1500 ppm. Reitz et al. (1988) also discussed the data and the model predictions for older mice (Schumann et al., 1982b). Reitz et al. suggested that increasing the size of the fat compartment could improve the ability of the model to predict results obtained from older and fatter mice.

The model proposed by Reitz et al. (1987, 1988) was used as the basis for modeling MC and for extensions that considered TCA kinetics (Figure IV-1). In essence, the MC PBPK model was linked to a single compartment model for TCA via the P-450 metabolism of MC. A certain proportion (PO) of MC metabolized in that manner was converted to TCA. The precursor in TCA production, trichloroethanol (TCOH), was ignored in this approach. TCA was eliminated from its volume of distribution according to the first-order rate, Ke. Work with trichloroethylene (for which TCA is also a metabolite) provided the basis for this representation of TCA and its link to the PBPK model of the parent compound (Fisher et al., 1990; Allen et al., 1990).

Other approaches to modeling TCA kinetics have been proposed. Nolan et al. (1984) presented a model for MC that explicitly considered TCOH, the precursor of TCA in MC metabolism. Their model included three physiologically based compartments for modeling MC kinetics (liver was lumped with other rapidly perfused tissues), a single volume of distribution for TCOH (into which the MC metabolized is introduced and from which TCOH is expired, eliminated, or metabolized to TCA), and a single volume of distribution for TCA (into which the TCOH metabolized is introduced and from which TCA is excreted).

Caperos et al. (1982) proposed a model similar to that of Nolan et al. (1984). Their model utilized first-order rate constants to describe in detail the transformation and elimination of TCOH and TCA. However, the manner in which these metabolites were considered to be distributed was not completely described. It was not stated what volumes of distribution were assumed for TCOH or for TCA, although it may be inferred that they were the same as those used in a similar representation of TCOH and TCA after TCE exposure (Fernandez et al., 1977).

The models of Nolan et al. (1984) and Caperos et al. (1982) were not considered further in this assessment. The approach to handling the distribution and kinetics of TCOH (a long-lived intermediate in TCA production) may be worth additional investigation. It is not clear at this time if adequate data are available to estimate the parameters required in such an approach in the case of rodents.

C. MODEL EXTENSION AND PARAMETER ESTIMATION

The proposed model (Figure IV-1) has a physiological basis for describing MC distribution, elimination, and metabolism. The model for MC was linked to a single-compartment representation for TCA kinetics. The estimates available from the literature (Reitz et al., 1987, 1988) for the human and mouse models are displayed in Table IV-1.

1. Mouse Parameter Estimates

The parameters suggested for mice by Reitz et al. (1988) differed slightly from those proposed by Reitz et al. (1987), most notably with respect to the metabolic rate constants, Vmaxc and Km. Both sets of parameter estimates were based on optimization of the model fit to rat data (Schumann et al., 1982a) with subsequent scaling to mice. The later report (Reitz et al., 1988) apparently considered an adjustment to the data from Schumann et al. (1982a) that was not considered in the earlier report (Reitz et al., 1987). The parameter values presented by Reitz et al. (1987, 1988) provided the starting points for selection of the parameter values used in this analysis.

Physiological and Partition Coefficient Parameter Estimates. The first step in the estimation of parameters to characterize the pharmacokinetics of MC and TCA in mice was the selection of initial values for the physiological parameters. Since no information concerning those parameters (other than body weights) was given in the literature (Holmberg et al., 1977; Schumann et al., 1982a, 1982b), values suggested as mouse reference values by Arms and Travis (1987) were used (Table IV-2). Comparing mouse values in Arms and Travis (1987) (Table IV-2) to the values in Reitz et al. (1987, 1988) (Table IV-1),

it can be observed that the Arms and Travis reference values differ from those used by Reitz et al. (1987, 1988) in the following ways:

- For the younger animals, the reference values for the liver and fat compartment volumes are larger and the reference value for the slowly perfused tissue volume is smaller than the corresponding volumes used by Reitz et al. (1987, 1988).
- For the older animals, the reference compartment volumes differ from those cited in Reitz et al. (1987, 1988) in that a larger proportion is allocated to the fat compartment and smaller proportions to the liver and rapidly perfused compartments. The reference slowly perfused compartment volume is between those cited in Reitz et al. (1987) and Reitz et al. (1988). The volumes given as reference values for older mice were derived by assuming that one half of the weight increase, from 29 to 40 grams (Schumann et al., 1982a, 1982b), was due to increased fat and the other half was attributable to increased volume of muscle and skin (slowly perfused tissues).
- The reference pulmonary ventilation rate is larger than that cited by Reitz et al. (1987, 1988); the reference cardiac output is smaller than that used by those authors.
- Corresponding to the larger reference fat compartment, the reference value for blood flow to the fat is also larger than used by Reitz et al. Reference values for flows to the slowly and rapidly perfused tissues are smaller than those used by Reitz et al. (1987, 1988). Flow to the liver is about the same.

Described below are the modifications to the values of the physiological parameters and partition coefficients that were made so as to obtain predictions from the mouse model that match, as closely as possible, the observations reported in Schumann et al. (1982a, 1982b) and Holmberg et al. (1977).

Holmberg et al. (1977) exposed male NMRI mice weighing between 25 and 30 grams to MC concentrations ranging from 100 to 10,000 ppm. They measured MC concentrations in the blood, liver, kidney, and brain during and following exposure, which lasted up to 24 hours. The concentrations in the brain were very close to those observed in the kidney; thus the kidney observations were used to characterize the rapidly perfused tissue concentrations.

These data were used to adjust the partition coefficients pla and pra (see Figure IV-1), which determine how much MC partitions into the liver and rapidly perfused tissues, respectively. The model predictions of liver and rapidly perfused tissue concentrations were matched to observed liver and kidney concentrations, respectively, and pla and pra were adjusted until adequate predictions of the experimental data were obtained for all the atmospheric concentrations tested. It was determined that the predictions of liver and rapidly perfused tissue concentrations were very insensitive to the values of the metabolic parameters, so it was considered adequate to concentrate solely on pla and pra in this fitting process. Both of the parameters were increased from the starting values provided by Reitz et al. (1987, 1988); pla was increased by a factor of 2.5 (to 21.5) and pra by a factor of 1.75 (to 15.1).

The data of Schumann et al. (1982a, 1982b) provided an opportunity to verify the revised estimates of pla and pra and to verify predictions of MC

concentration in the fat as well. Schumann et al. (1982a) exposed male B6C3F1 mice (average weight of 29 grams) to radiolabeled MC at concentrations of either 150 or 1500 ppm for 6 hours. Measurements obtained by these authors included blood and tissue concentrations (the latter determined by the radioactivity), amounts of MC exhaled unchanged during the 72 hours after the end of exposure, and the amount of MC metabolized and appearing as CO_2, urinary or fecal metabolites, or contaminants of the body at the time of sacrifice. Schumann et al. (1982b) conducted the same experiment (using only the 1500 ppm exposure level) but used older mice (about 18 months old, averaging 40 grams in weight) that had been exposed either once or repeatedly since they were 9 to 10 weeks old.

When pla and pra were set at the values suggested by analysis of the Holmberg et al. (1977) data (21.5 and 15.1, respectively), the predicted concentrations in the kidneys (actually concentrations in the rapidly perfused tissues) matched the observed values extremely well. On average, the predictions of liver concentrations were about 32% higher than observed concentrations. This was a result of considerable overestimation of liver concentration in the young mice and slight underestimation of that concentration in the older mice. For these comparisons, we assumed that all of the radioactivity observed in the tissues at the end of the exposure was due to MC itself.

The predictions of the MC concentrations in fat were not close to the concentrations experimentally observed. It was determined that altering the partition coefficient, pfa, did not produce predictions of fat concentrations that matched the Schumann et al. observations for all their experiments (young and old mice, low and high exposures, repeated or single exposure). However,

reducing qfc, the proportion of the cardiac output directed to the fat, while at the same time keeping the sum of qfc and qsc fixed at 24%, did result in predictions of fat concentrations close to those observed (Table IV-3). It was not necessary to alter pfa when qfc was set to 3% of the cardiac output. This value of qfc, and the corresponding value of qsc (21%), were used to obtain all of the predictions presented in Table IV-3.

When these revised physiological parameter values were used in conjunction with the revised estimates of pla and pra, the model predictions for the Holmberg et al. (1977) experiments were as shown in Figure IV-2. No substantial changes in the predictions of liver and richly perfused tissue concentrations were seen when qfc and qsc were modified.

Adjustment of qfc and qsc resulted in better predictions of the observed data. However, it should be noted that no data concerning MC concentrations in muscle and skin (slowly perfused tissues) were available. Thus the adjustment of qfc and qsc improves the prediction of fat concentrations but we could not determine if the resulting changes in slowly perfused tissue concentrations were consistent with actual behavior.

It is also worth noting the pattern of liver concentration predictions in relation to the observations of those concentrations by Schumann et al. (1982a, 1982b). The value of pla estimated on the basis of the results of Holmberg et al. (1977) yielded estimates of liver concentration that were too high for the younger mice but too low for the older mice (Table IV-3). This was the case even when the exposure of the young and the old mice was the same. It may be possible that some parameters other than fat and slowly perfused tissue volumes are changing with age and affecting liver concentration. It was observed that changing qlc (the proportion of the

cardiac output directed to the liver) did not alter the estimates of MC liver concentrations or amounts metabolized. It may be the case that the composition of the liver may be changing with age, e.g., the livers may be getting more fatty, thus increasing the amount of MC retained in the liver. At this time, no conclusions could be reached concerning this issue. All of the subsequent work assumed age-independent partitioning.

Metabolic Parameter Estimation. Starting from the values suggested by Reitz et al. (1987, 1988), the parameters determining the metabolism of MC (Vmaxc and Km) were revised on the basis of mouse data. The relevant data were obtained from Schumann et al. (1982a, 1982b).

Schumann et al. estimated the amounts of MC metabolized following the exposures to 150 or 1500 ppm. There are, however, some problems with the manner in which the data were collected that limit their usefulness for estimating values of the metabolic parameters. The estimates of metabolism presented by Schumann et al. were based on exhalation of labeled CO_2, urinary and fecal radioactivity, and radioactivity remaining in the carcass at the time of sacrifice. All of these samples were obtained only after exposure had stopped; therefore, the observed amount metabolized did not include metabolites eliminated during the exposure. On the other hand, some of the urinary and fecal activity, and probably also some of the CO_2 activity, would have been due to metabolism that occurred during exposure, but for which the elimination had not yet occurred when the animals were transferred to metabolism cages. Thus, the observed amount metabolized was less than the total amount metabolized, but somewhat greater than the amount metabolized post-exposure.

Given that the data from Schumann et al. (1982a, 1982b) are the only data available on metabolism in mice, they were used. It was assumed that the Schumann et al. observations of amounts metabolized were close to (only slight overestimates of) the amount metabolized post-exposure. The goal in the estimation of Vmax and Km was to get predictions of the amounts metabolized after the end of exposure that were close to, without exceeding, the values reported by Schumann et al.

That goal was at least partially satisfied. The value of Vmaxc was increased to 2.05 and Km was unchanged; the resulting predictions of amounts metabolized are displayed in Table IV-4; they are shown in relation to the observations of Schumann et al. (1982a, 1982b). Increasing Vmaxc had some impact on the tissue concentrations as of the end of exposure. In fact, the predicted concentrations showed somewhat closer agreement with the observed values. The ratio of predicted to observed liver concentrations, for example, was 1.27, on average, as opposed to the average ratio of 1.32 with the preliminary value of Vmaxc (Table IV-3). Table IV-4 also shows observed and predicted amounts of MC exhaled unchanged. The estimates of the amount of MC exhaled were quite good for the younger mice but tended to be low for the older mice.

Figure IV-3 displays the observed and predicted concentrations of MC in venous blood following exposure of the young mice to 150 or 1500 ppm MC. The agreement between observed and predicted concentrations was very good, especially at the lower exposure level.

TCA Parameter Estimation. The parameter estimation discussed above concerned the distribution and elimination of MC and can be considered as a refinement of the model parameter estimates that were already in existence

(Reitz et al., 1987, 1988). To complete the model that we have proposed, i.e., the model that also includes tracking of TCA, additional parameters were estimated. These parameters describe the proportion of metabolized MC that becomes TCA, the volume of distribution for TCA, and the rate of TCA elimination.

Unfortunately, there were no experimental data directly related to the concentration or elimination of TCA after exposure to MC in mice. Results obtained for TCE and PERC exposure as well as the results of Schumann et al. (1982a, 1982b) were used as guidance for the estimation of parameter values discussed here. Schumann et al. (1982a, 1982b) reported data from mice for urinary metabolites and radioactivity remaining in the carcass at the time of sacrifice.

Fisher et al. (1990) obtained estimates for the parameters defining TCA kinetics in mice following TCE exposure (Table IV-5). The female mice appeared to have a smaller volume of distribution for TCA and a higher rate of TCA elimination than did the male mice.

In the case of PERC, a much greater proportion of metabolized parent appeared as TCA than in the case of TCE, and the apparent rate of TCA elimination was somewhat smaller than in the case of TCE. The same volume of distribution for TCA was determined to be acceptable for both parent compounds (see Volume III, Part 2 of this document).

Based on the observations for TCE and PERC, the following assumptions were made when estimating the TCA parameters suitable for MC exposure.

The volume of distribution (with scaling constant Vdc) was assumed to be equal to that used in the cases of TCE and PERC. This assumption

implies that the volume of distribution for TCA is independent of parent compound.

- Trichloroethanol (TCOH) is a precursor for TCA production in the case of MC, but not in the case of PERC. Since TCOH is a long-lived intermediate and will produce TCA even after MC has been eliminated (Muller et al., 1974; see also Volume II, Part 1), the apparent rate of TCA elimination may be smaller when TCOH is a precursor than in cases in which TCA is the first stable product of the metabolism of the parent. In other words, the apparent rate of TCA elimination (being the difference between the rate of production and the rate of disappearance) may be smaller when the rate of production continues to be positive for longer periods of time, e.g., when TCOH persists. That is the case with MC but not with PERC (EPA, 1984; Dekant et al., 1986). Thus, the values for the elimination rate considered when fitting the data of Schumann et al. (1982a, 1982b) were constrained to be less than that for PERC, i.e., Kec was assumed to be less than 0.025.
- A value for PO in the case of MC was difficult to determine simply on the basis of the PO values for TCE and PERC. The requirement of TCOH as a precursor for TCA production following MC exposure would make PO more similar to that for TCE than to that for PERC. However, in TCE metabolism, some TCA is produced without TCOH as a precursor (tending to make the PO for TCE greater than that for MC). Conversely, the conversion of TCE to short-lived intermediates which can yield products other than chloral hydrate, the precursor for both TCA and TCOH production, is an "extra" step in the production of TCOH and TCA not evident in MC metabolism. For MC, TCOH is the first product of MC P-450

metabolism (EPA, 1984). This extra step tended to make PO for TCE less than that for MC. Overall, a PO for MC was assumed to be similar (but not necessarily equal) to that for TCE.

Given the constraints and suggestions just presented, the data from Schumann et al. (1982a, 1982b) were used to more completely define Kec and PO. The reported values of the MC-equivalents remaining in the carcass at the time of sacrifice (72 hours after the end of exposure) represented lower bounds for the predicted amount of TCA remaining in an animal. They were lower bounds because some of the tissues were apparently removed before the carcass was examined for radioactivity. It was assumed that all other products of MC metabolism were present in the carcass in negligible quantities at the time of sacrifice.

Conversely, the amounts of MC-equivalents in the urine represented upper bounds for the amount of TCA eliminated. They were upper bounds because other products, notably TCOH, would also be eliminated in the urine and contribute to the observed radioactivity there. However, given that the collection of urine began only upon completion of exposure (i.e., after 6 hours from the beginning of exposure) and given the fact that TCOH appears to have a shorter half-life than TCA, substantial amounts of TCOH may have been eliminated in the urine before the end of exposure, thereby not contributing to the measured radioactivity. If that is the case, then the observed MC-equivalents in urine may not represent as much of an upper bound as might otherwise be the case.

Table IV-6 displays the bounds provided by the Schumann et al. (1982a, 1982b) data and the model predictions when PO=0.07 and Kec=0.01. Those parameter values were determined by inspection to best satisfy the constraints

imposed by consideration of the results obtained with TCE and PERC and those imposed by the Schumann et al. data. Note that the predicted radioactivity in the carcass of older mice exposed to 1500 ppm was lower than the presumed lower bound. However, increasing PO made the urinary excretions for the other exposures (150 or 1500 ppm in younger mice) too large. Decreasing Kec made the urinary output for the older mice even smaller when it was already well below the presumed upper bound.

Based on this discussion, the values of the parameters PO and Kec were selected to be 0.07 and 0.01, respectively. These values were used in subsequent PBPK modeling of mice in order to characterize the kinetics of MC and TCA. The true values of PO and Kec are quite uncertain and will remain so until more pertinent data obtained from MC exposure experiments are available.

The predictions for TCA in urine ranged from about 27 to 78%, with an average of about 56%, of the observed urinary radioactivity. These percentages were somewhat larger than those observed in studies of rats (Eben and Kimmerle, 1974; Koizumi et al., 1982; Hake et al., 1960; Ikeda and Ohtsuji, 1972). This may be a species difference or it may reflect the fact that not all urine was collected (i.e., not during exposure) as discussed above.

2. Human Parameter Estimates

Initial Parameter Estimates. The estimates of the human parameter values presented by Reitz et al. (1987, 1988) are displayed in Table IV-1. Changes to those parameters are listed in Table IV-7; the values in the first column of Table IV-7 represent the initial values used to define a human MC/TCA PBPK model.

The volumes of the compartments given by Reitz et al. were similar to those listed as reference values for humans by Arms and Travis (1987); slightly more of the body was composed of liver and fat according to the values used by Reitz et al. (and slightly less was rapidly or slowly perfused tissue) in comparison to the Arms and Travis values. Similarly, blood flows to compartments were similar to those reported by Arms and Travis, although the Reitz et al. values implied that somewhat more blood flowed to the rapidly perfused tissues and to the fat, and somewhat less to the liver and to the slowly perfused tissues, in comparison to the Arms and Travis values. The reference values from Arms and Travis for those parameters were the values used in subsequent modeling.

The resting alveolar ventilation rate (with scaling constant qpc) and the resting cardiac output rate (scaling constant qcc) were adjusted in light of data from Astrand et al. (1973), Monster et al. (1979) and Hattis et al. (1986). The resting value for qpc estimated from those sources is 17.3. The data presented in Astrand et al. (1973) regarding ventilation rate and cardiac output rate for varying levels of activity (exercise) suggested an equation of the form

$$(1) \qquad qcc = 12.3 + (0.278 * qpc),$$

so that the qcc corresponding to the resting qpc was 17.1.

Alternative partition coefficients were also selected for initial model fitting. The values were uniformly higher than those used by Reitz et al. (compare Table IV-1 and the initial values listed in Table IV-7). In the case of the liver and the rapidly perfused tissue compartments, the ratios of the

tissue/air coefficients to the blood/air coefficient (which determine partitioning of MC between the tissues and the blood) were very similar when using the Reitz et al. values or the initial values listed in Table IV-7. The initial values entailed higher partitioning into the fat and slowly perfused tissues in comparison to the partitioning determined by the Reitz et al. values.

The blood/air partition coefficient (pb) value of 2.63 selected for initial model fitting was an average of values reported by Caperos et al. (1982; pb = 4.35), Gargas et al. (1989; pb = 2.53), Morgan et al. (1970; pb = 1.4), Nolan et al. (1984; pb = 1.57), and Sato and Nakajima (1979; pb = 3.3). The remaining tissue-to-air coefficients were taken from Caperos et al. (1982). Although it was not entirely clear from the description in Caperos et al. (1982), it appeared that those coefficients were estimated using human tissues.

The parameters that determined the kinetics of TCA were PO (the proportion of MC metabolized that ends up as TCA), Vdc (the scaling constant for the volume of distribution for TCA), and Kec (the scaling constant for the first-order rate of elimination of TCA). The estimates of these parameters shown as initial values in Table IV-7 were obtained as follows.

Allen et al. (1990) estimated the proportion of metabolized trichloroethylene (TCE) converted to TCA by considering all the pathways by which TCA could be produced. One of those pathways was the conversion of TCE to TCOH (through an epoxide, chloral, and chloral hydrate) and the subsequent oxidation of TCOH to TCA. The last step in that pathway was the important aspect for MC because all TCA produced from MC has TCOH as an intermediate (EPA, 1984). Allen et al. (1990) estimated that 27% of TCOH was converted to

TCA (the remainder being excreted or conjugated). Thus, an initial value for PO was 0.27. (This value assumes that all MC metabolized was converted to TCOH.)

The volume of distribution scaling constant, Vdc, was also determined for humans by Allen et al. (1990). They presented a regression equation for Vdc as follows

$$(2) \qquad Vdc = 0.341 - (0.0034 * bw),$$

where bw was body weight in kg. This equation accounted for the apparently nonconstant proportion of the total body size into which TCA distributes. This equation was observed to work well when describing TCA concentrations following TCE and TCA exposures. The same equation was used for Vdc in the case of MC. It was assumed that TCA distributes the same (into the same space) regardless of parent compound.

The initial value for Kec (0.035) was that suggested by Nolan et al. (1984) based on their observation that the average half-life for TCA in blood was 76 hours. This value was derived from the following equations, which hold when elimination is a first-order process:

$$(3a) \qquad Ke = -\ln(0.5)/t_{1/2};$$

$$(3b) \qquad Kec = Ke/bw^{-0.3};$$

where $t_{1/2}$ is the half-life and bw is body weight.

The apparent rate of TCA elimination was affected by the compound that was administered (Muller et al., 1974). Thus it was difficult to estimate

elimination rates that should hold over a variety of exposure conditions. However, the value of 0.035 could be compared to estimates discussed in Allen et al. (1990) following TCOH exposure and those presented in Volume III, Part 2 of this document for PERC exposure.

Considering that TCOH is the immediate precursor for TCA production following MC exposure, the rate of TCA elimination used in the model should be similar to that observed after TCOH administration. Muller et al. (1974) estimated the half-life of TCA in plasma after TCOH administration to be 65.39 hours (corresponding to a Kec value of 0.038). However, rates of TCA elimination following TCA administration estimated by Muller et al. (1974) were larger than similar estimates derived from other sources (Paykoc and Powell, 1945). Using the average of all such estimates (Kec = 0.0396) and correcting for the difference between half-lives observed after TCA administration and TCOH administration (Muller et al., 1974; the ratio is 0.77), a lower estimate for Kec was 0.031.

On the other hand, the rate of TCA elimination following PERC exposure (Volume III, Part 2) was estimated to correspond to a Kec of 0.045 for humans. TCOH is not a precursor for TCA production following PERC exposure and, in fact, no long-lived precursor for TCA exists when PERC is the parent compound (Dekant et al., 1986). The lack of a persistent source of TCA production following PERC exposure implies that the apparent rate of TCA elimination should be greatest following PERC exposure. In light of the estimates derived for TCE and PERC, the initial estimate of Kec (0.035) derived from Nolan et al. (1984) was a reasonable starting value.

The initial value of PU, the proportion of eliminated TCA that appears as TCA in the urine, was also taken from the derivation in Allen et al. (1990)

following TCE exposure. The value of this parameter should be independent of the parent compound. That is, the elimination of TCA, whether by metabolism or excretion, should not be influenced by the compound that produces TCA. Thus, the initial value for PU, 0.934, should be as suitable for use in the MC/TCA model as in the TCE/TCA model of Allen et al. (1990).

Human data and parameter revisions. The data used to revise and validate the human model have been obtained from the peer-reviewed literature. Of particular value were the reports of Nolan et al. (1984), Monster et al. (1979), Caperos et al. (1982), and Stewart et al. (1969). Also utilized in the comparisons of predictions and observations were data from Astrand et al. (1973), Imbriani et al. (1988), Mackay et al. (1987), and Seki et al. (1975).

As in the case of the development of the mouse model, the parameters defining MC distribution and metabolism were examined first. Model predictions of tissue concentrations and exhaled breath concentrations were relatively sensitive to the values of those parameters and could be compared to the data from the literature in order to refine values of such parameters.

Nolan et al. (1984) and Monster et al. (1979) presented extensive data with respect to MC concentrations in blood and exhaled breath. On the basis of those results, the values of pfa and psa for humans were modified to be 200 and 18.3, respectively. Those changes allowed much closer prediction of the blood and breath concentrations, especially 24 to 170 hours from the start of exposure (exposure lasted for 4 to 6 hours) (Figures IV-4, IV-5 and IV-6). With those values, the blood concentration was somewhat overpredicted during exposure when compared to the data of Nolan et al. (1984) (Figure IV-4) when atmospheric concentration was either 35 or 350 ppm. However, predicted blood concentration during exposure was in close agreement with the results of

Monster et al. (1979) (Figures IV-5 and IV-6); the atmospheric concentrations in that study were 72 and approximately 145 ppm. Moreover, the observations presented by Mackay et al. (1987) were predicted extremely well by the model (Figure IV-7); one of the atmospheric concentrations used in that study was 350 ppm.

The predictions of the model with revised values of pfa and psa were verified with the data from Caperos et al. (1982) and Stewart et al. (1969). The data and model predictions are shown in Figures IV-8 and IV-9. The agreement between the observed and predicted values is very good.

Next, attention was focussed on the metabolism of MC and, in particular, its conversion to TCA. Monster et al. (1979) and Nolan et al. (1984) presented results directly relevant to the estimation of the parameters defining that process. Those authors reported concentrations of TCA in the blood of individuals exposed to MC (72 and 142-145 ppm for Monster et al.; 35 and 350 ppm for Nolan et al.). In addition, "indirect" data that were relevant to the estimation of such parameters were available from Monster et al. (1979), Nolan et al. (1984), Stewart et al. (1969), Caperos et al. (1982), and Seki et al. (1975). Those documents reported excretion of TCA in the urine.

The data from Seki et al. (1975) were obtained from occupationally exposed workers, where the atmospheric concentrations of TCA were quite low, ranging from 4.3 to 53.4 ppm. The Seki et al. (1975) report was important for at least two reasons. First, it documented the fate of MC in chronically exposed individuals. Chronic exposures are those that are most often of concern in the context of regulation. Second, the results suggested the possibility of saturation of MC metabolism even at the low atmospheric

concentrations to which the workers were exposed. This saturation was suggested by the fact that a 5.7-fold increase in atmospheric concentration (4.3 to 24.6 ppm) was accompanied by a 4-fold increase in TCA excretion (0.6 to 2.4 mg/hr); a further 2.2-fold increase in atmospheric concentration (24.6 to 53.4 ppm) elicited only a 1.5-fold increase in TCA excretion (2.4 to 3.6 mg/hr).

The first step that was taken to revise the model parameters defining MC metabolism and TCA kinetics was to investigate modifications to Km and Vmaxc in light of the results presented by Seki et al. (1975). If no other parameters were adjusted, a value of 0.17 for Vmaxc and a value of 0.25 for Km yielded predictions of urinary TCA excretion that were in good agreement with the observations:

Concentration (ppm)	TCA Excretion (mg/L) Observed	Predicted
4.3	0.6	0.6
24.6	2.4	2.4
53.4	3.6	3.8

The observed urinary TCA values reported above were taken from the afternoon in "the latter half of the week" (Seki et al., 1975); therefore, the model predictions were obtained from a simulated Friday afternoon on the third week of an occupational exposure scenario (8 hr/day, 5.5 days per week).

The alternate values of Km and Vmaxc (0.25 and 0.17, respectively) were tested against the results of the other experiments cited above. It was determined that the value of Km (0.25) appeared to be too small, since predicted TCA concentrations were often too large in comparison with the observations from those experiments. The value of Km was increased to 0.30

and, as shown in Figures IV-10 through IV-13, the predictions obtained were generally in good agreement with the experimental observations. The increase in the value of Km did not greatly affect the predicted TCA excretion for the Seki et al. (1975) exposure scenario; the predicted values changed from those given in the table above to 0.5, 2.2, and 3.4 mg/L for MC concentrations of 4.3, 24.6, and 53.4 ppm, respectively.

The predicted concentrations of TCA in blood were greater than the observed concentrations for the 35 ppm MC exposure tested by Nolan et al. (1984) (Figure IV-10). However, the predictions for their 350 ppm exposure were much closer to the corresponding observations, and the predicted rates of TCA excretion in the urine were in excellent agreement with the observed rates at that dose level. Even for the 35 ppm exposure, the predicted excretion rates were only slightly high; they matched the observed rates better than the predicted TCA blood concentrations at that exposure level matched the corresponding observed blood concentrations.

For the exposures studied by Monster et al. (1979) (72 and 142-145 ppm), the model predictions of TCA concentrations in the blood were very close to the corresponding observations, especially for the higher exposure levels (Figure IV-11). Urinary TCA output, however, was overestimated at all exposure levels.

The urinary TCA data from Caperos et al. (1982) were well predicted by the model (Figure IV-12). The slight overestimation of TCA in the urine for the 72 ppm exposure level represented a better prediction of the observations than that obtained for the observations of Monster et al. (1979) at the same exposure level. As opposed to the general overprediction of TCA excretion [when compared to the observations of Caperos et al. (1982) and to those of

the other studies discussed above], urinary TCA during four days of exposure to 507 ppm (7 hours per day) was underpredicted when compared to the results of Stewart et al. (1969) (Figure IV-13).

In general, the predictions of the model as defined by the revised parameter set (consisting of altered values for psa, pfa, Vmaxc, and Km; see Table IV-7) were very close to the corresponding observations obtained from the peer-reviewed literature. The model with the revised parameter set was considered to reasonably represent the kinetics of MC and TCA in humans. The revised parameter set was, therefore, considered to provide the "final" estimates of the parameters; the model with those parameter values was used for subsequent risk assessment.

The alternate Km value of 0.30 was substantially smaller than those suggested by Reitz et al. (1987, 1988) (see Table IV-1 and Table IV-7). The alternate value for Vmaxc (0.17) was also slightly smaller than the values suggested by those authors. Thus, use of these alternate values in the model implied that MC metabolism saturated at a much lower dose than would be predicted by earlier models (Reitz et al., 1987, 1988) but that the rate of metabolism under saturation conditions was smaller than predicted by earlier models.

D. RISK ASSESSMENT

The PBPK models developed above for mice and humans yielded estimates of delivered doses (dose surrogates) that may be related to the production of liver tumors. Although such tumors have not been observed to be statistically significantly related to MC exposure (NCI, 1977a; Quast et al., 1988), liver

tumors were found in mice after exposure to TCE and PERC (NCI, 1976, 1977b; NTP, 1986, 1990). The presumed liver carcinogen common to all three compounds is TCA. The potential for human liver cancer risk associated with exposure to MC can be evaluated in light of the mouse results for TCE, PERC, and MC and the TCA-based dose surrogates estimated by the PBPK models of those compounds.

Thus, the following procedure was followed. The mouse PBPK model for each of the compounds was used to estimate dose surrogates corresponding to the experimental doses in the bioassays conducted on that compound. The dose surrogate estimates were matched to the observed liver tumor response rates for dose-response modeling. Predictions of the dose surrogate values corresponding to extra risks of 10^{-6} and 10^{-3} were predicted by the dose-response model. Using the human MC PBPK model, the exposure levels corresponding to the predicted dose surrogate values were determined.

The dose surrogates that were considered for an assessment of liver cancer risks were average daily values of 1) the amount of TCA produced per liver volume, and 2) the area under the TCA concentration curve. Both of these dose surrogates are of interest because of their potential relationship to mechanisms of liver tumor production. TCA may be considered to be a liver carcinogen that acts through its effect on peroxisome proliferation (see Volume II, Part 2, Section C). Such proliferation has been observed in response to xenobiotics only in the liver.

TCA production per liver volume provided a measure of TCA specific to the liver, prior to its introduction into the systemic circulation. If the action of TCA that induces tumor production is relatively rapid, then the long-term kinetics of TCA may not be as important as the rate at which it is being produced. Alternatively, such a dose surrogate could be relevant if TCA

does not easily return to the target sites (within the liver or within the cell) once it has left the liver.

Area under the TCA concentration curve was based on the concentration of TCA in its volume of distribution. Thus, this measure was not associated specifically with the liver. However, it did provide an indication of the persistence of TCA; unlike TCA production, area under the concentration curve provides a measure relevant to products, such as TCA, that are long-lived and are therefore present for extended periods of time. It is assumed with a dose surrogate such as area under the concentration curve that the reactions responsible for tumor induction could occur at any time that TCA is present.

Gavage exposures (NCI, 1976, 1977a, 1977b) were represented in the PBPK model as direct inputs to the liver that lasted for 2 hours, at which time all administered dose was absorbed. The linearized multistage modeling approach that is the standard dose-response procedure for regulatory agencies (e.g., the EPA) was used.

Dose-response data for MC are displayed in Table IV-8. Data for TCE and PERC are shown in Tables II-2-1 (Volume II, Part 2) and III-2-7 (Volume III, Part 2), respectively. As discussed in Volume II, Part 2A, male mice were not analyzed for TCE.

The results of the risk estimation based directly on MC carcinogenicity bioassays are presented in Table IV-9. The results are expressed in terms of concentrations (atmospheric or drinking water) that are associated with two levels of extra risk to humans (10^{-6} and 10^{-3}) when exposures to those concentrations last the entire lifetime. Drinking water exposure was represented in the PBPK model as continuous input to the liver, assuming 100%

absorption and an intake of 2 liters per day. The body weight assumed for the calculations was 70 kg.

The concentrations associated with either of the two levels of risk depended on the dose surrogate selected for low-dose and species-to-species extrapolation. The assessment based on the dose surrogate representing the amount of TCA produced per liver volume yielded the smaller concentrations. Area under the TCA concentration curve yielded larger concentrations for the specified risk levels than did TCA production because TCA elimination was estimated to be more rapid in humans than in mice (Kec in humans was 0.045 and in mice it was 0.025). This more rapid elimination compensated for the smaller volume of distribution (per body weight) in humans than in mice. A smaller volume of distribution tends to increase TCA concentrations (for the same amount metabolized) thus tending to increase risk when estimated on a TCA concentration basis.

For comparison, the atmospheric concentrations associated with 10^{-6} and 10^{-3} risk when the standard EPA analysis was completed (without consideration of pharmacokinetic differences, and assuming mice and humans are equally sensitive when dose is expressed as mg/surface area/day) were determined to be 2.9×10^{-3} ppm and 2.9 ppm, respectively. The drinking water concentrations determined by that method were 108 µg/L and 1080 µg/L, respectively. Use of either of the dose surrogates for the estimation of risk decreased the "allowable" atmospheric or drinking water concentrations.

The concentrations estimated to be associated with the levels of risk discussed above should be considered to be lower bounds. That is, higher concentrations may yield risks no greater than those given. This is the case because, in accordance with standard regulatory procedure, the doses reported

were the 95% lower bounds predicted by the multistage model. In addition, TCA may be acting through its effects on peroxisomes. Humans may be less susceptible to the peroxisome proliferating effects of TCA (Elcombe, 1985). Section 2B of Volume II of this report discussed the issues associated with peroxisome proliferation, including the PBPK modeling extensions that may be necessary to derive appropriate dose surrogates and the use of such information in dose-response modeling.

E. COMPARISON WITH TCE AND PERC

In the bioassays of MC (NCI, 1977a; Quast et al., 1988) a significant increase in the rate of hepatocellular tumors in either male or female mice was not reported. The risk estimates derived above were based on the male mouse results obtained from NCI (1977a), despite the fact that the rates did not reach statistical significance.

It is also possible to support the use of bioassay results obtained using TCE and PERC for the estimation of human liver cancer risks associated with MC exposure. That use is reasonable if the production and distribution of TCA is what determines the liver cancer risk associated with exposure to all three compounds. In that case, the parent compound is irrelevant, as long as one has the ability to estimate TCA production and distribution.

Table IV-10 displays the TCA-AUC dose surrogate values estimated to correspond to experimental doses used in bioassays of TCE, PERC, and MC. The TCA-AUC estimates were obtained by using the mouse PBPK models corresponding to the administered compound (the models discussed in Volumes II and III and in this volume). Also shown in Table IV-10 are the observed additional risks

for the dose groups, where additional risk was based on the difference in liver tumor rates between the dose group and the corresponding control. Data in Table IV-10 suggest a fairly strong correlation between the TCA-AUC dose surrogate and risk.

The major "outliers" in terms of that correlation appear to be results for female mice exposed to TCE (the group with TCA-AUC = 350 and risk = 0.32 and possibly the group with TCA-AUC = 341 and risk = 0.23). It may be the case that the estimates of some of the TCE model parameters for female mice are in error. It may be the case that other metabolites of TCE (perhaps DCA or an epoxide) are contributing to the liver tumor response following TCE exposure. It may be the case that the result from the NTP (1990) study (which used only a single dose group in addition to controls) is an outlier with respect to the liver tumor response rate observed. All of these possibilities could account for the apparent deviation of some of the TCE results from the pattern observed with the other chemicals and other TCE dose groups, i.e., they would make the TCA-AUC dose appear to be more potent as a measure of liver carcinogenic potential than might actually be the case.

The TCA-AUC values associated with the MC exposures were uniformly low in comparison with those associated with the TCE or PERC experimental doses. Thus, the lack of an observable liver tumor response in the MC bioassays is consistent with TCA-AUC as a relevant measure of the carcinogenic potency. That is, the doses administered in the MC bioassays were not high enough to produce the amount of TCA which would result in significantly increased liver tumor response rates under the conditions of the bioassay.

Table IV-11 shows the TCA-AUC values associated with a risk of 10^{-6} estimated by the multistage model applied to the bioassays discussed above.

With the exception of the NTP (1990) study of TCE, for which the TCA-AUC dose corresponding to 10^{-6} risk is about an order of magnitude lower than the others, the results are very consistent. Again, this suggests that TCA-AUC may be a reasonable dose surrogate on which to base risk estimates. That suggestion follows from the considerations given above and the fact that, across species and across sexes, consistent risk estimates were obtained with that dose surrogate.

An important consequence of the results shown in Table IV-11 is that the use of the TCE or PERC bioassays for estimation of human MC liver cancer risks would not substantially alter the results shown in Table IV-9, when TCA-AUC is the dose surrogate. That is, the human atmospheric and drinking water concentrations estimated to correspond to 10^{-3} and 10^{-6} risks would be only slightly smaller (by no more than a factor of 4, based on the results shown in Table IV-11 if the result for TCE obtained from the NTP (1990) is ignored). Thus, the results of using the MC bioassay (Table IV-9) appear consistent with the other evidence, despite the fact that the liver tumor response rates following MC exposure were not significantly increased.

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Table IV-1

Parameter Values from Published Literature
for Mouse and Human PBPK Models

	Mouse		Human	
	Reitz (1987)[a]	Reitz (1988)[b]	Reitz (1987)[a]	Reitz (1988)[b]
Compartment volumes (L/kg bw)				
vlc	0.04	0.04	0.026	0.031
vrc	0.05	0.05	0.064	0.037
vsc	0.79 (0.67)[c]	0.78 (0.64)[c]	0.635	0.611
vfc	0.04 (0.16)[c]	0.04 (0.18)[c]	0.195	0.231
Alveolar and total cardiac flow rates ($L/hr/kg^{.74}$)				
qpc	17.3 (17.1)[c]	17.3	12.6	13.2
qcc	17.3 (17.1)[c]	17.3	12.6	13.2
Compartment blood flows (% of cardiac output)				
qlc	24.0	24.0	24.0	24.0
qrc	56.0	53.0	53.0	49.0
qsc	18.0	21.0	14.0	18.0
qfc	2.0	2.0	9.0	9.0
Partition coefficients (unitless)				
pb	10.8	10.8	2.53	2.53
pla	8.6	8.6	8.6	8.6
pra	8.6	8.6	8.6	8.6
psa	3.15	3.15	3.15	3.15
pfa	263	263	263	263
Metabolic constants				
Vmaxc ($mg/hr/kg^{.7}$)	0.265	0.419	0.265	0.419
Km (mg/L blood)	6.43	5.75	6.43	5.75

[a] Parameter values from Reitz et al. (1987).
[b] Parameter values from Reitz et al. (1988).
[c] Values in parentheses are for older mice weighing about 40 g. The younger mice weighed about 29 g on average.

Table IV-2

Reference Values for Physiological Parameters in Mice

Parameter	Young mice[a] (bw = 0.025 kg)	Older mice[b] (bw = 0.040 kg)
Compartment volumes (L/kg bw)		
vlc	0.055	0.04
vrc	0.05	0.036
vsc	0.70	0.65
vfc	0.10	0.21
Alveolar and total cardiac flow rates ($L/hr/kg^{.74}$)		
qpc	22.9	22.9
qcc	15.9	15.9
Compartment flow rates (% of cardiac output)		
qlc	25.0	25.0
qrc	51.0	51.0
qsc	15.0	15.0
qfc	9.0	9.0

[a] Arms and Travis (1987).

[b] All but the compartment volumes are from Arms and Travis (1987). The volumes were derived by assuming that the increase in body weight (40 g - 29 g) was distributed only to the fat and slow perfused tissues, half the body weight gain to each compartment.

Table IV-3

Observed and Predicted Concentrations of MC in Mouse Tissues

Study	Concentration in:	Observed[b]	Predicted[a] Vmaxc=0.419	Predicted[a] Vmaxc=2.05
Schumann et al., 1982a, young mice				
150 ppm	liver	10.1 ± 1.0	16.8	15.3
	kidney	10.0 ± 0.9	11.9	11.2
	fat	117 ± 16	191	180
1500 ppm	liver	84.2 ± 19	175	172
	kidney	147 ± 77	123	121
	fat	2161 ± 239	1947	1956
Schumann et al., 1982b, older mice				
1500 ppm, single exposure	liver	239 ± 40	167	165
	kidney	117 ± 25	118	116
	fat	1414 ± 204	1420	1407
1500 ppm, repeated exposure[c]	liver	199 ± 55	167	165
	kidney	109 ± 29	118	117
	fat	1172 ± 301	1441	1427

[a] Predicted values were obtained with parameters as shown in Table 2, except that qsc = 21% and qfc = 3%. In addition, pb = 10.8, pla = 21.5, pra = 15.1, psa = 3.15, pfa = 263, Km = 5.75, and Vmaxc is as shown.

[b] Observed means ± 2 x S.E.M.

[c] Predicted values were obtained at the end of the last simulated exposure, where the simulation was set for five 6-hour exposures per week, for 2 weeks.

Table IV-4

Observed and Predicted Metabolism and Exhalation of MC

Study	Variable (mg)	Observed	Predicted
Schumann et al., 1982a, young mice			
150 ppm	Amount metabolized	0.09	0.08
	Amount exhaled	0.58	0.53
1500 ppm	Amount metabolized	0.16	0.51
	Amount exhaled	5.2	6.1
Schumann et al., 1982b, older mice			
1500 ppm, single exposure	Amount metabolized	1.1	1.2
	Amount exhaled	19	12
1500 ppm, repeated exposure[a]	Amount metabolized	1.3	1.2
	Amount exhaled	15	12

[a] Predicted values were obtained at the end of the last simulated exposure, where the simulation was set for five 6-hour exposures per week, for 2 weeks.

Table IV-5

Parameters Describing TCA Kinetics in Mice

Parameter	TCE[a]	PERC[b]	MC[c]
PO (%)	7 (10, 18)[d]	52	7
Vdc (L/kg bw)	M: 0.24 F: 0.176	0.24 ---	0.24 ---
Kec ($hr^{-1}/kg^{-.3}$)	M: 0.043 F: 0.104	0.025 ---	0.01 ---

[a] From Fisher et al. (1990), which includes a model for TCE and TCA in male and female mice.
[b] From Volume III, Part 2 of this report, which includes a model for PERC and TCA in male mice.
[c] The parameter values selected for MC, as discussed in the text.
[d] In parentheses are PO values for males and females, respectively, at the lowest TCE dose levels, as derived by Fisher et al. (1990).

Table IV-6

Observed and Predicted Variables
Relevant to Estimation of Kec and FO

Variable	Exposure Level (ppm)	Observed	Predicted
Radioactivity in carcass[a] (μmol-eq)	150	0.01[b]	0.04
	1500	0 [b]	0.95
	1500[c]	0.20[b]	0.14
Radioactivity in urine[d] (μmol-eq)	150	0.43[e]	0.28
	1500	0.82[e]	0.64
	1500[c]	3.74[e]	0.98

[a] Radioactivity remaining in the carcass at the time of sacrifice, 72 hours after the end of exposure.

[b] These values represent lower bounds for model predictions (from Schumann et al., 1982a, except as noted).

[c] The observed value is the average for older mice exposed to 1500 ppm of MC either once or repeatedly (Schumann et al. 1982b).

[d] Radioactivity in urine excreted from the end of exposure to the time of sacrifice.

[e] These values represent upper bounds for model predictions (from Schumann et al., 1982a, except as noted).

Table IV-7

Initial and Final Parameter Values
Defining the Human MC/TCA PBPK Model

Parameter	Value	
	Initial	Final
Compartment Volumes (L/kg bw)		
vlc	0.026	0.026
vrc	0.05	0.05
vsc	0.62	0.62
vfc	0.19	0.19
Alveolar and total cardiac flow rate ($L/hr/kg^{.74}$)		
qpc	17.3 (resting)	17.3 (resting)
qcc	17.1 (resting)	17.1 (resting)
Compartment blood flows (% of cardiac output)		
qlc	0.26	0.26
qrc	0.44	0.44
qsc	0.25	0.25
qfc	0.05	0.05
Partition coefficients (unitless)		
pb	2.63	2.63
pla	9.1	9.1
pra	9.1	9.1
psa	6.1	18.3
pfa	373.0	200.0
Metabolic constants		
Vmaxc ($mg/hr/kg^{.7}$)	0.419	0.17
Km (mg/L blood)	5.75	0.30
TCA kinetic constants		
PO (%)	27	27
Vdc (L/kg bw)	0.341-(0.0034*bw)	0.341-(0.0034*bw)
Kec ($hr^{-1}/kg^{-.3}$)	0.035	0.035
PU (%)	93.4	93.4

Table IV-8

Dose-Response Data for Bioassays of MC in Mice

Bioassay	Doses[a] Experimental	Doses[a] $[TCA]_p$	Doses[a] TCA-AUC	Liver Tumor Response Rate[b]
Quast et al. (1988)	0	0	0	29/50
Inhalation	150	23.52	200.30	22/50
Male	500	38.88	331.22	28/50
	1500	53.41	454.93	24/50
Quast et al. (1988)	0	0	0	13/50
Inhalation	150	23.52	200.30	10/50
Female	500	38.88	331.22	10/50
	1500	53.41	454.93	7/50
NCI (1977a)	0	0	0	0/15
Gavage	2406	34.19	283.94	0/47
Male	4813	42.75	354.00	4/40
NCI (1977a)	0	0	0	0/18
Gavage	2406	34.94	270.11	0/48
Female	4813	43.53	337.43	0/50

[a] Experimental doses are reported in mg/kg body weight for gavage studies and in ppm air for concentration inhalation studies. $[TCA]_p$ is amount of TCA produced per liver volume (mg/L); TCA-AUC is area under the TCA concentration curve (mg*hr/L).

[b] Number of mice with hepatocellular adenomas or carcinomas per number of mice examined.

Table IV-9

Inhalation and Drinking Water Risk Assessment
Results: Mice Exposed to MC

Bioassay	Risk[c]	Associated Dose Surrogate Values[a] $[TCA]_p$	Associated Dose Surrogate Values[a] TCA-AUC	Estimated Human Air Concentrations (ppm)[b] $[TCA]_p$	Estimated Human Air Concentrations (ppm)[b] TCA-AUC
NCI (1977a) Gavage Male	1E-03	8.00E-01	6.59E0	1.4E0	2.6E0
	1E-06	7.99E-04	6.59E-03	1.3E-03	2.4E-03

Bioassay	Risk[c]	Estimated Human Water Concentrations (mg/L)[d] $[TCA]_p$	Estimated Human Water Concentrations (mg/L)[d] TCA-AUC
NCI (1977a) Gavage Male	1E-03	1.7E+01	3.2E+01
	1E-06	1.6E-02	2.9E-02

[a] The values of the dose surrogates estimated from the bioassay to correspond to the stated level of risk.

[b] The atmospheric concentrations to which humans would have to be exposed for a lifetime in order to obtain average daily dose surrogate values equaling those corresponding to the stated level of risk. Thus, the atmospheric concentrations are those estimated by each bioassay and dose surrogate combination to yield the stated level of risk.

[c] Extra risks [(P(d) P(0))/(1-P(0))].

[d] The drinking water concentrations to which humans would have to be exposed for a lifetime in order to obtain average daily dose surrogate values equaling those corresponding to the stated level of risk. Thus, the drinking water concentrations are those estimated by each bioassay and dose surrogate combination to yield the stated level of risk.

Table IV-10

Rank Ordering of Observed Risks from Bioassays of Mice Exposed to TCE, PERC, and MC

Sex	Additional Risk[a]	TCA-AUC	Chemical-Study
Female	.68	2862	PERC-NTP, 1986
	.32	350	TCE-NTP, 1990
	.30	2248	PERC-NCI, 1977b*
	.30	1632	PERC-NCI, 1977b*
	.26	1741	PERC-NTP, 1986
	.23	341	TCE-NCI, 1976
	.09	549	TCE-Bell et al. 1978
	.08	266	TCE-NCI, 1976
	.03	431	TCE-Bell et al. 1978
	.01	153	TCE-Bell et al. 1978
	.00	337	MC-NCI, 1977a
	.00	270	MC-NCI, 1977a
	-.06	331	MC-Quast et al. 1988
	-.06	200	MC-Quast et al. 1988
	-.12	455	MC-Quast et al. 1988
Male	.54	1971	PERC-NCI, 1977b
	.47	2648	PERC-NTP, 1986
	.44	2655	PERC-NCI, 1977b*
	.29	1667	PERC-NTP, 1986
	.08	354	MC-NCI, 1977a
	.00	284	MC-NCI, 1977a
	-.02	331	MC-Quast et al. 1988
	-.10	455	MC-Quast et al. 1988
	-.14	200	MC-Quast et al. 1988

[a] Additional risk is the observed rate of response (hepatocellular adenoma or carcinoma) in the dose group minus the observed rate of response in the corresponding control group.

* Dose groups marked with an asterisk experienced significantly lower rates of survival compared to the corresponding control group. Thus, the additional risk values may be underestimated if the reduced survival masked late appearing tumors.

Table IV-11

Estimates of the TCA-AUC
Doses Corresponding to 10^{-6} Extra Risk,
From Bioassays of Mice Exposed to TCE, PERC, and MC[a]

Sex	Chemical - Study	TCA-AUC
Female	PERC- NTP, 1986	5.3×10^{-3}
	NCI, 1977b	3.4×10^{-3}
	TCE - NTP, 1990	4.9×10^{-4}
	Bell et al., 1978	4.0×10^{-3}
	NCI, 1976	1.7×10^{-3}
Male		
	PERC- NTP, 1986	1.8×10^{-3}
	NCI, 1977b	2.2×10^{-3}
	MC - NCI, 1977a	6.6×10^{-3}

[a] 95% lower bounds on dose corresponding to risk of 10^{-6}, based on linearized multistage model fit to the response rates using the TCA-AUC dose surrogates corresponding to the experimental dosing patterns.

Figure IV-1
MC/TCA Pharmacokinetic Model

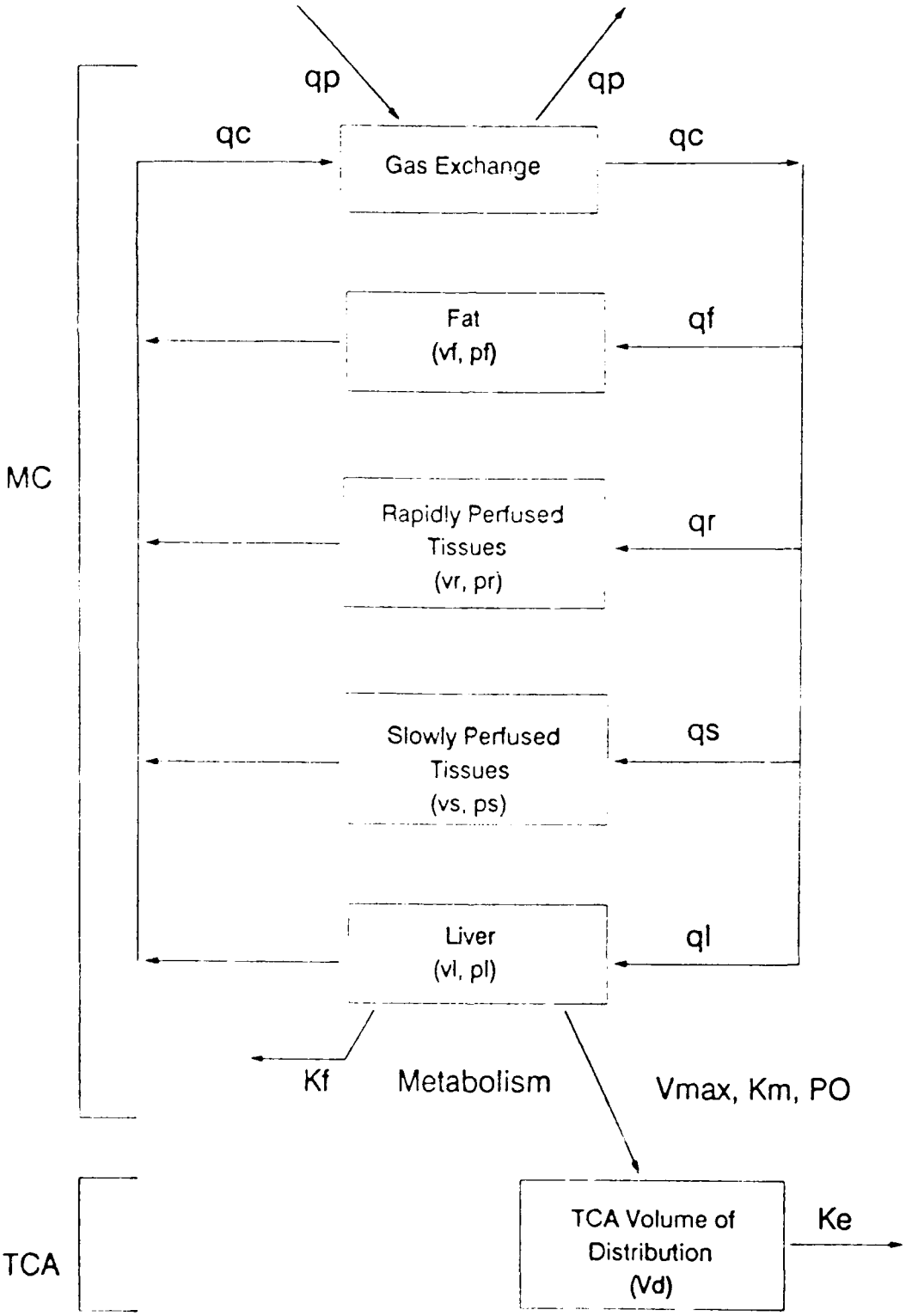
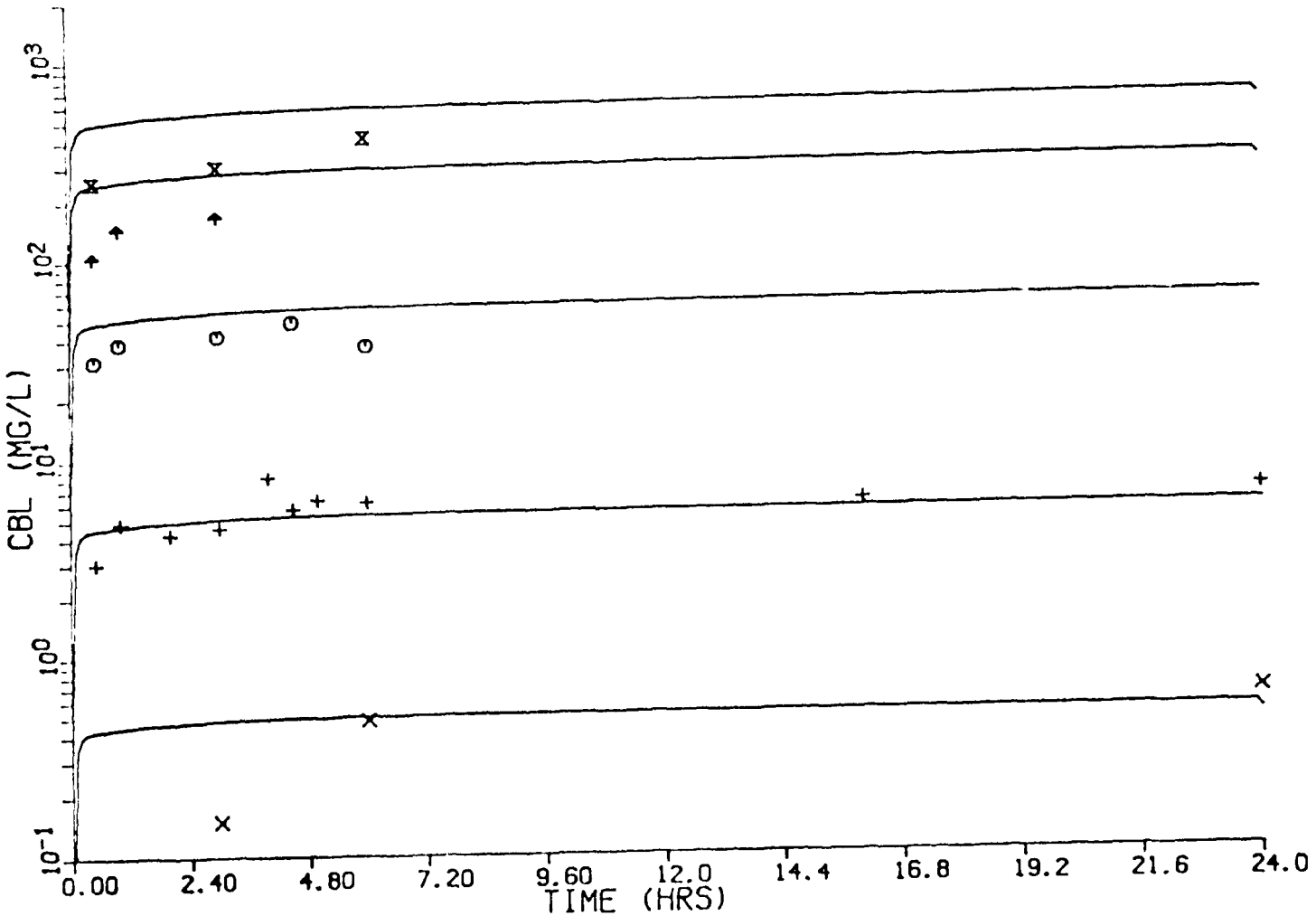


Figure IV-2a

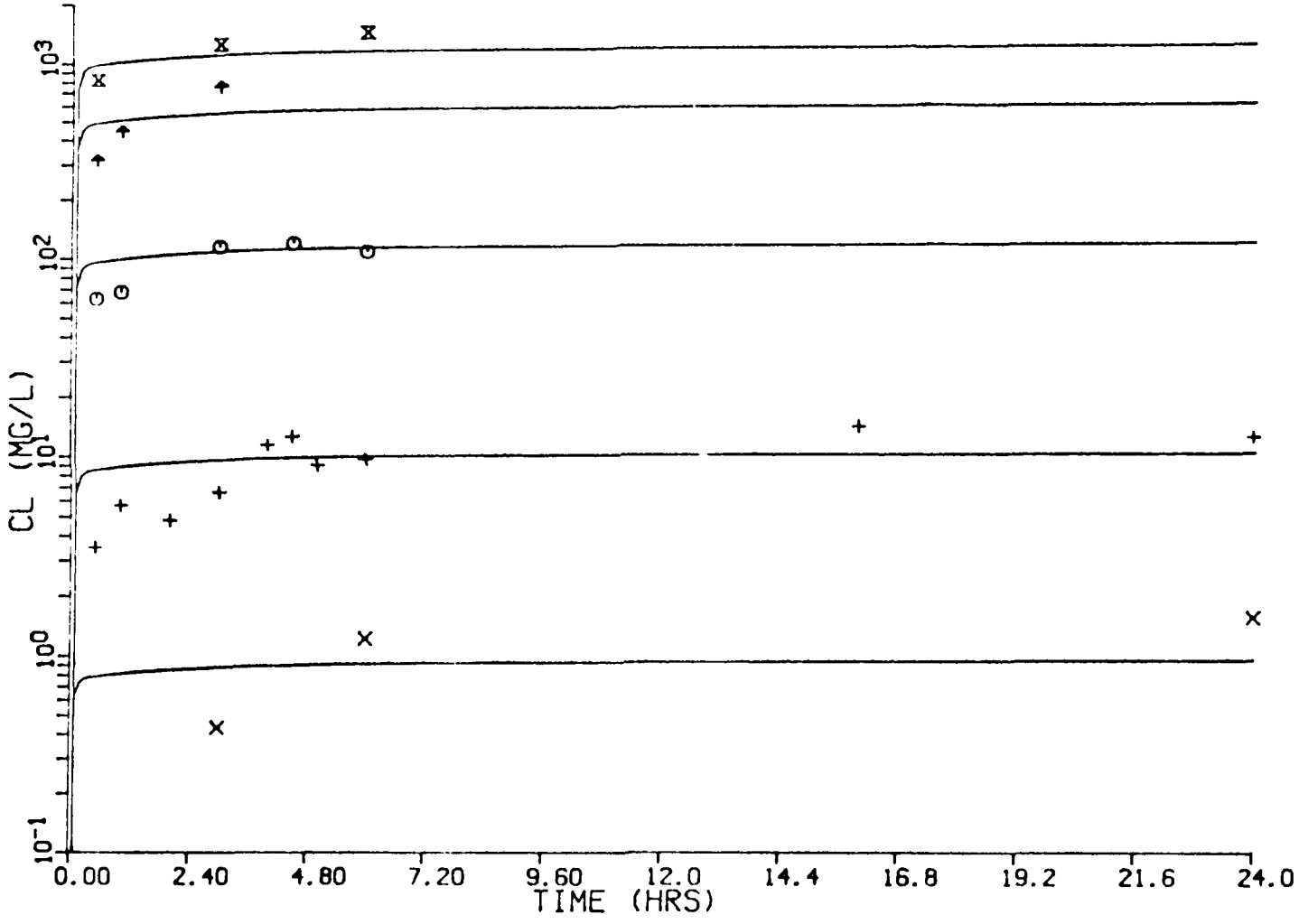
Observed and Predicted MC Concentrations in Mice



Observed concentrations from Holmberg et al. (1977) for blood during exposure to 10 (x), 100 (+), 1000 (Ⓞ), 5000 (ǃ), or 10000 ppm (ẍ) Solid lines represent model predictions.

Figure IV-2b

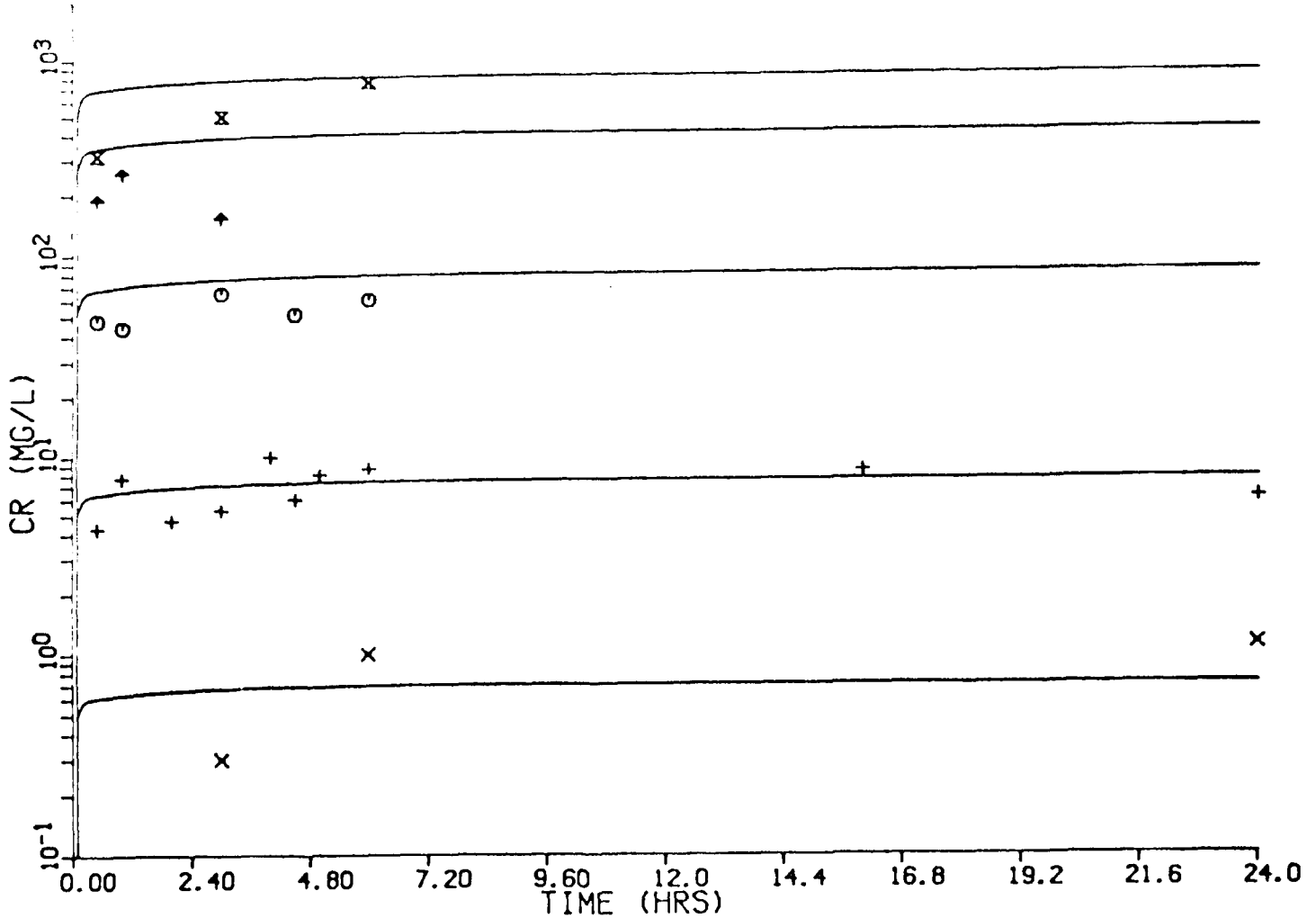
Observed and Predicted MC Concentrations in Mice



Observed concentrations from Holmberg et al. (1977) for liver during exposure to 10 (x), 100 (+), 1000 (Ⓞ), 5000 (ǃ), or 10000 ppm (x̲). Solid lines represent model predictions.

Figure IV-2c

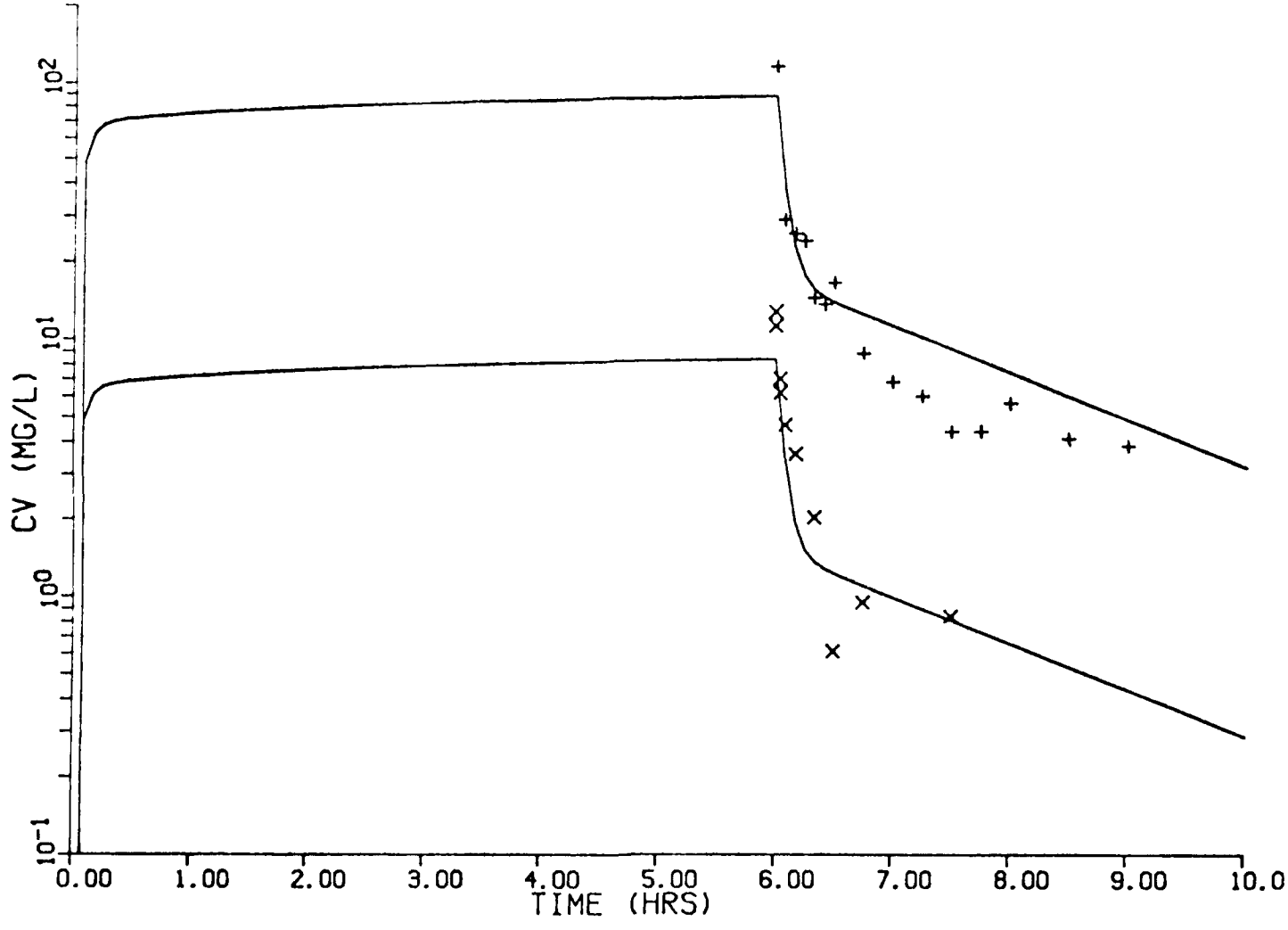
Observed and Predicted MC Concentrations in Mice



Observed concentrations from Holmberg et al. (1977) for kidneys during exposure to 10 (x), 100 (+), 1000 (Ⓞ), 5000 (!), or 10000 ppm (ⓧ). Solid lines represent model predictions.

Figure IV-3

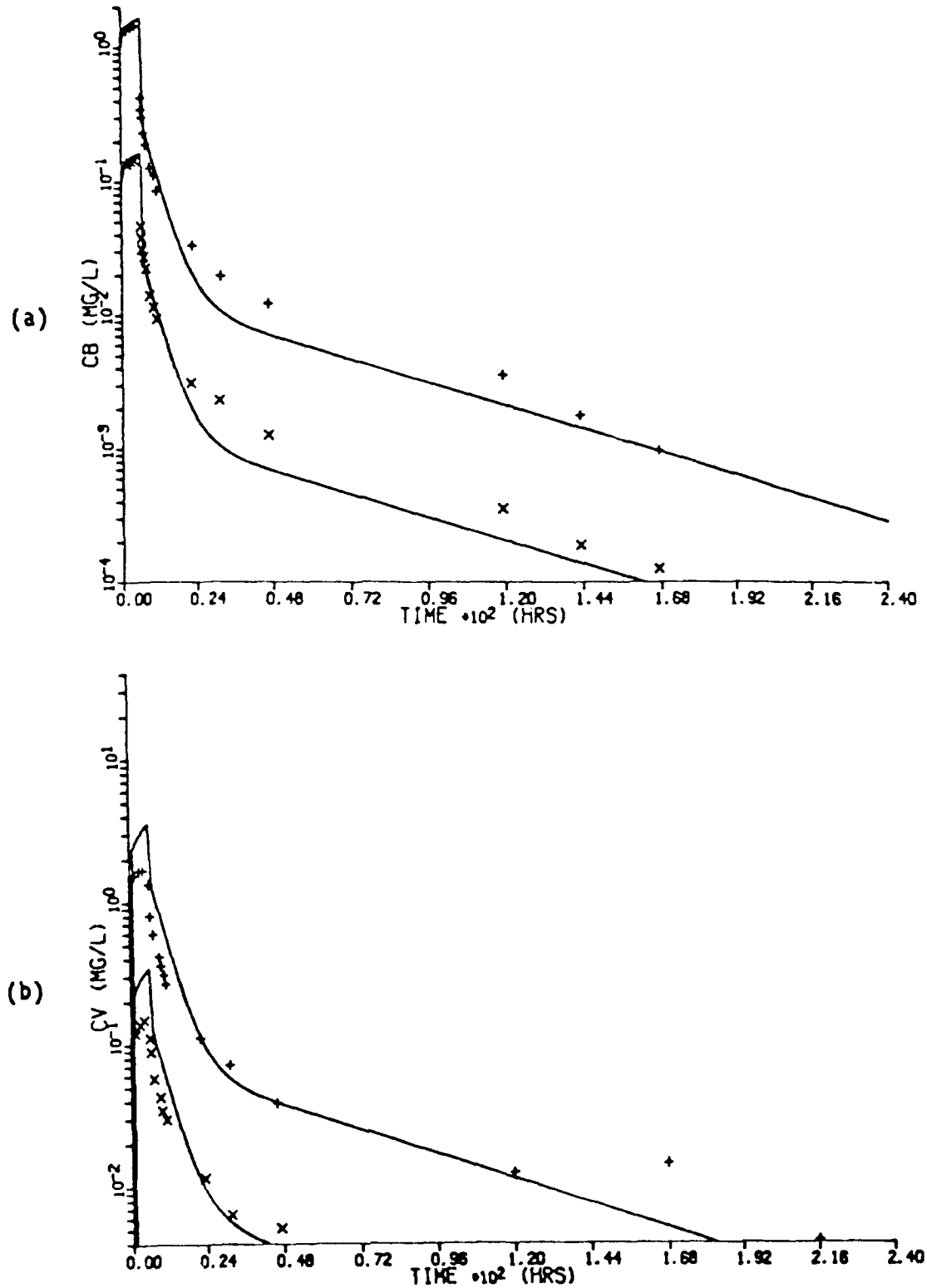
Observed and Predicted Venous Blood MC Concentrations in Mice



Observed concentrations from Schumann et al. (1982a) for venous blood concentrations following six-hour exposures to 150 ppm (x) or 1500 ppm (+). Solid lines represent model predictions.

Figure IV-4

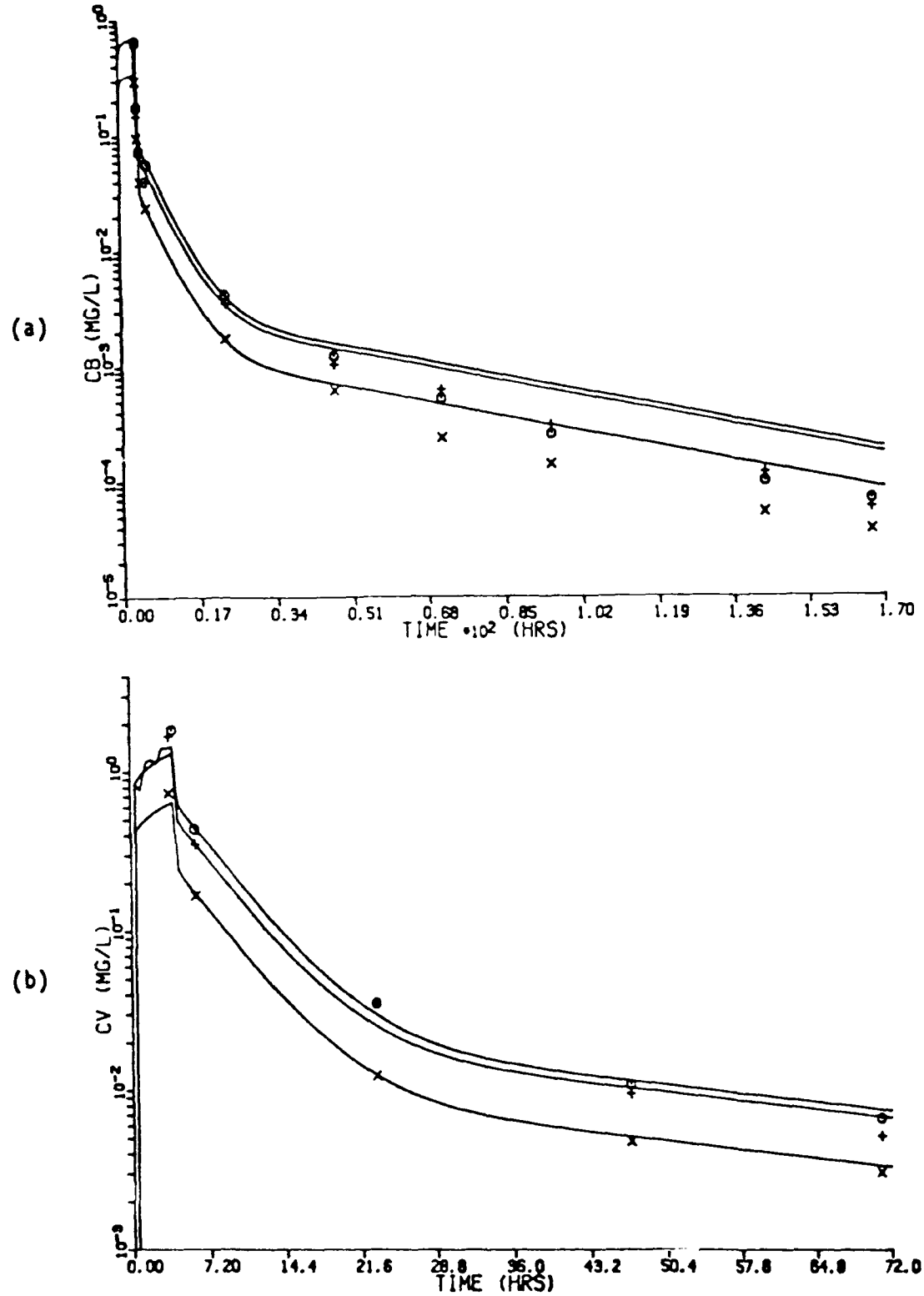
Observed and Predicted MC Concentrations in Humans (Nolan et al., 1984)



Observed concentrations from Nolan et al (1984) for exhaled breath (a) and venous blood (b) during and following six-hour MC exposures to 35 ppm (x) or 350 ppm (+). Solid lines represent model predictions.

Figure IV-5

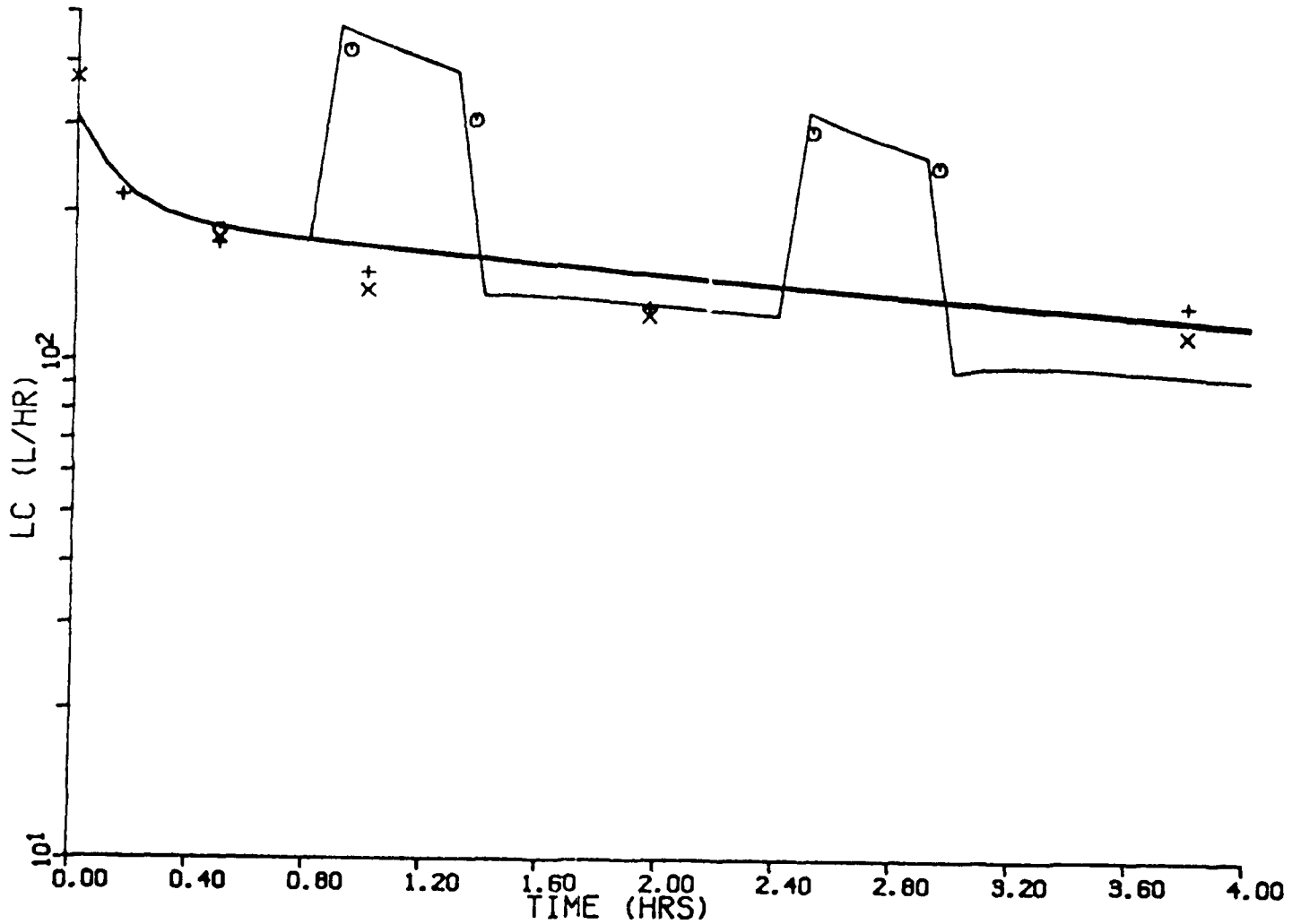
Observed and Predicted MC Concentrations
in Humans (Monster et al. 1979)



Observed concentrations for exhaled breath (a) and venous blood (b) (Monster et al., 1979). Subjects were exposed while resting to 72 ppm (x) or 144 ppm (+), or with exercise periods of 15 minutes alternating with longer periods of rest (142 ppm, ⏱). Solid lines represent model predictions.

Figure IV-6

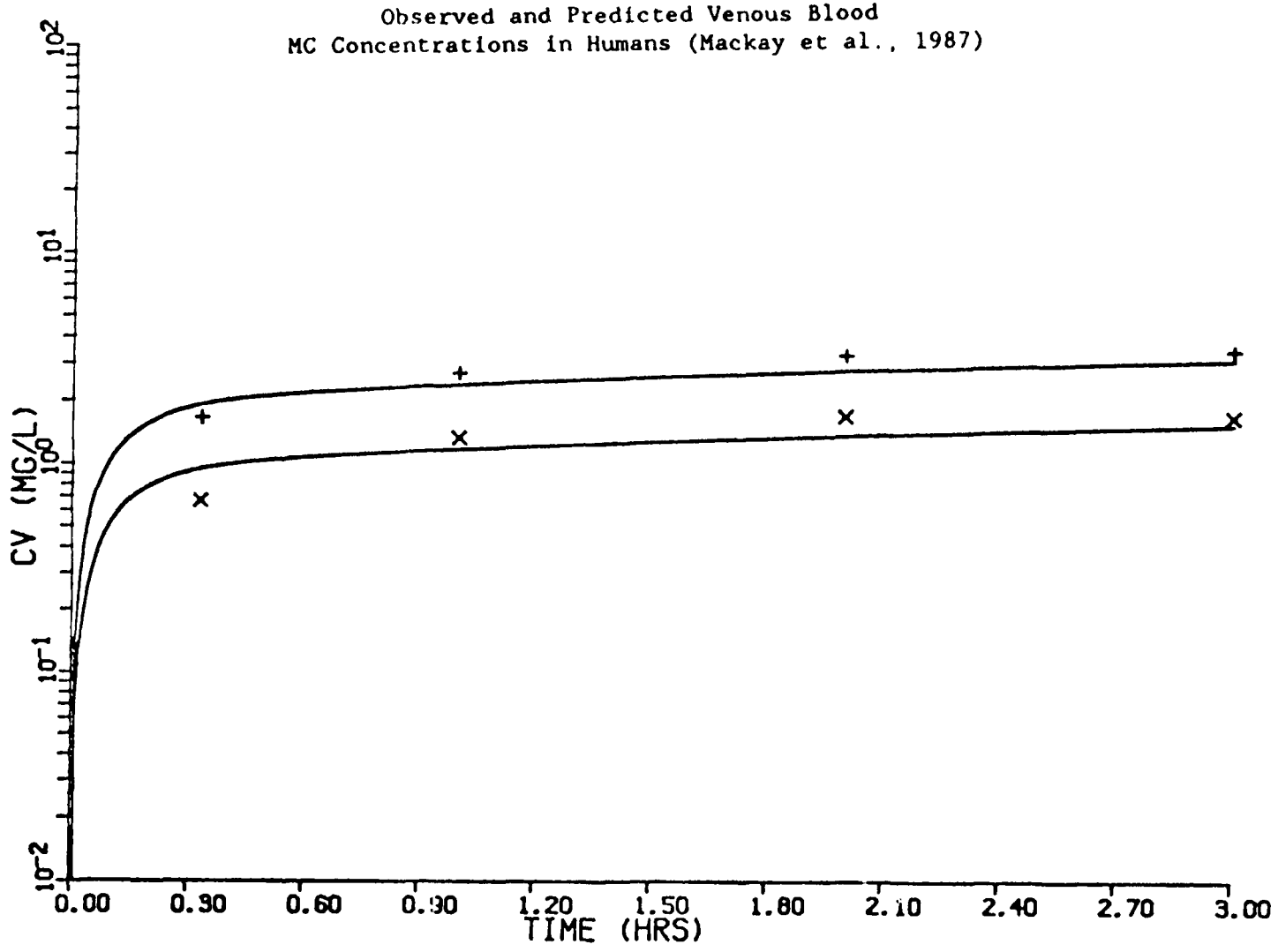
Observed and Predicted Lung Clearance in Humans (Monster et al. 1979)



Observed lung clearance during four-hour exposures (Monster et al., 1979). Subjects were exposed while resting, to 72 ppm (x) or 144 ppm (+), or with exercise periods of 15 minutes alternating with longer periods of rest (142 ppm, ⊙). Solid lines represent model predictions.

Figure IV-7

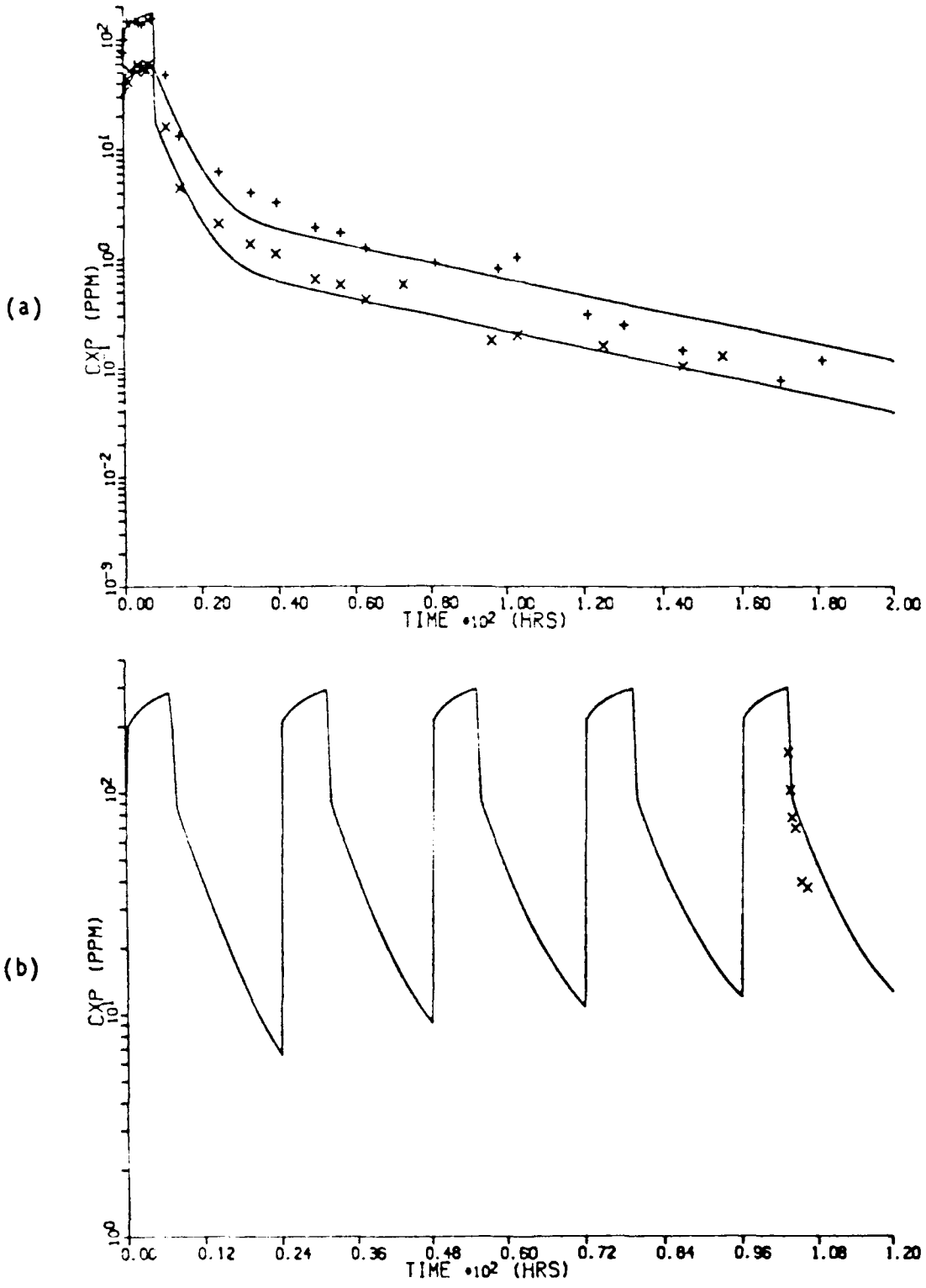
Observed and Predicted Venous Blood
MC Concentrations in Humans (Mackay et al., 1987)



Observed venous concentrations from Mackay et al. (1987) during exposure to 175 ppm (x) or 350 ppm (+). Solid lines represent model predictions.

Figure IV-8

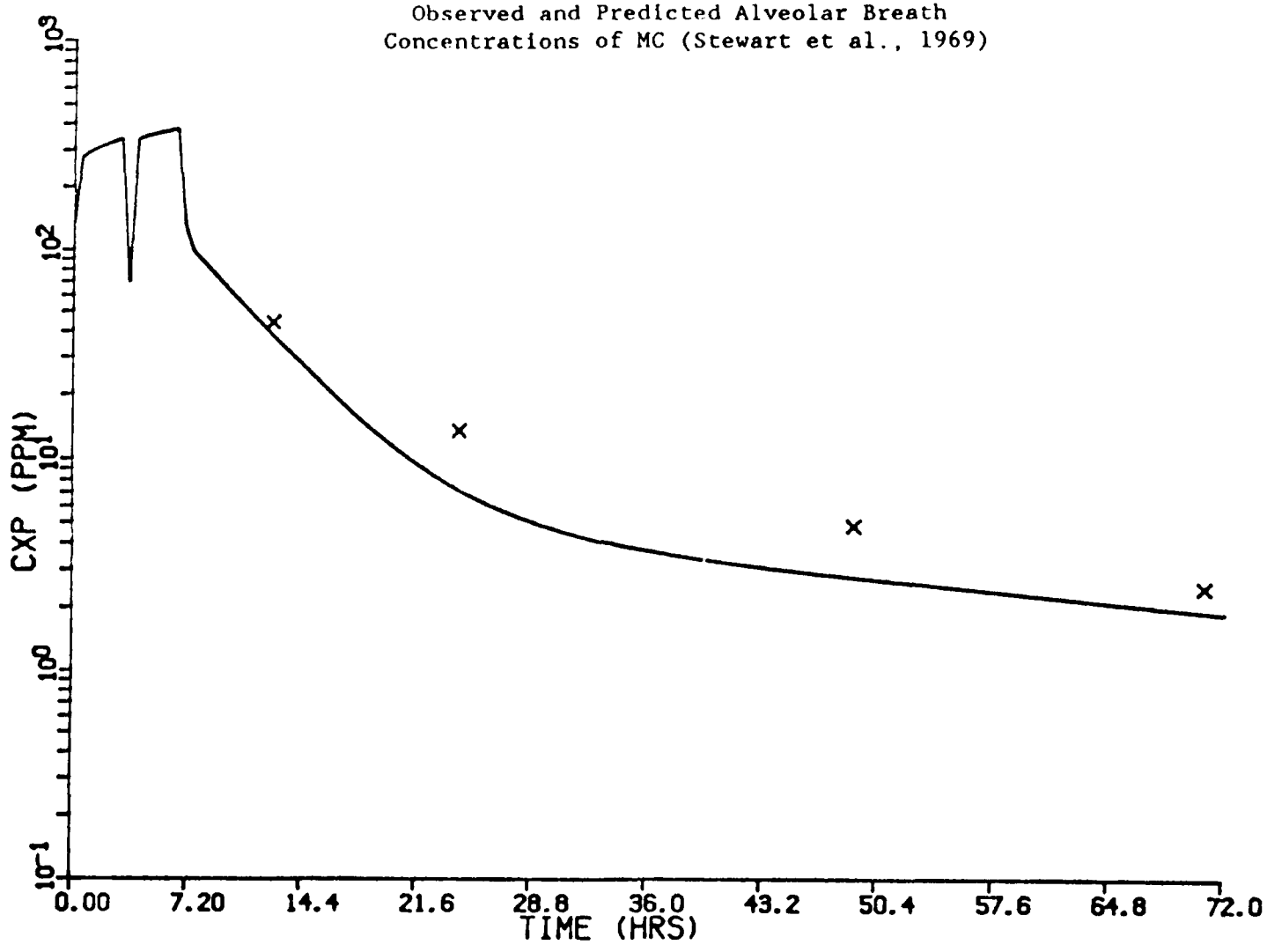
Observed and Predicted Alveolar Breath Concentrations of MC (Caperos et al., 1982)



Observed concentrations from Caperos et al. (1982): (a) during and following eight-hour exposures to 72 ppm (x) or 213 ppm (+); (b) following the fifth of five 7.5-hour, 350 ppm exposures (x). Solid lines represent model predictions.

Figure IV-9a

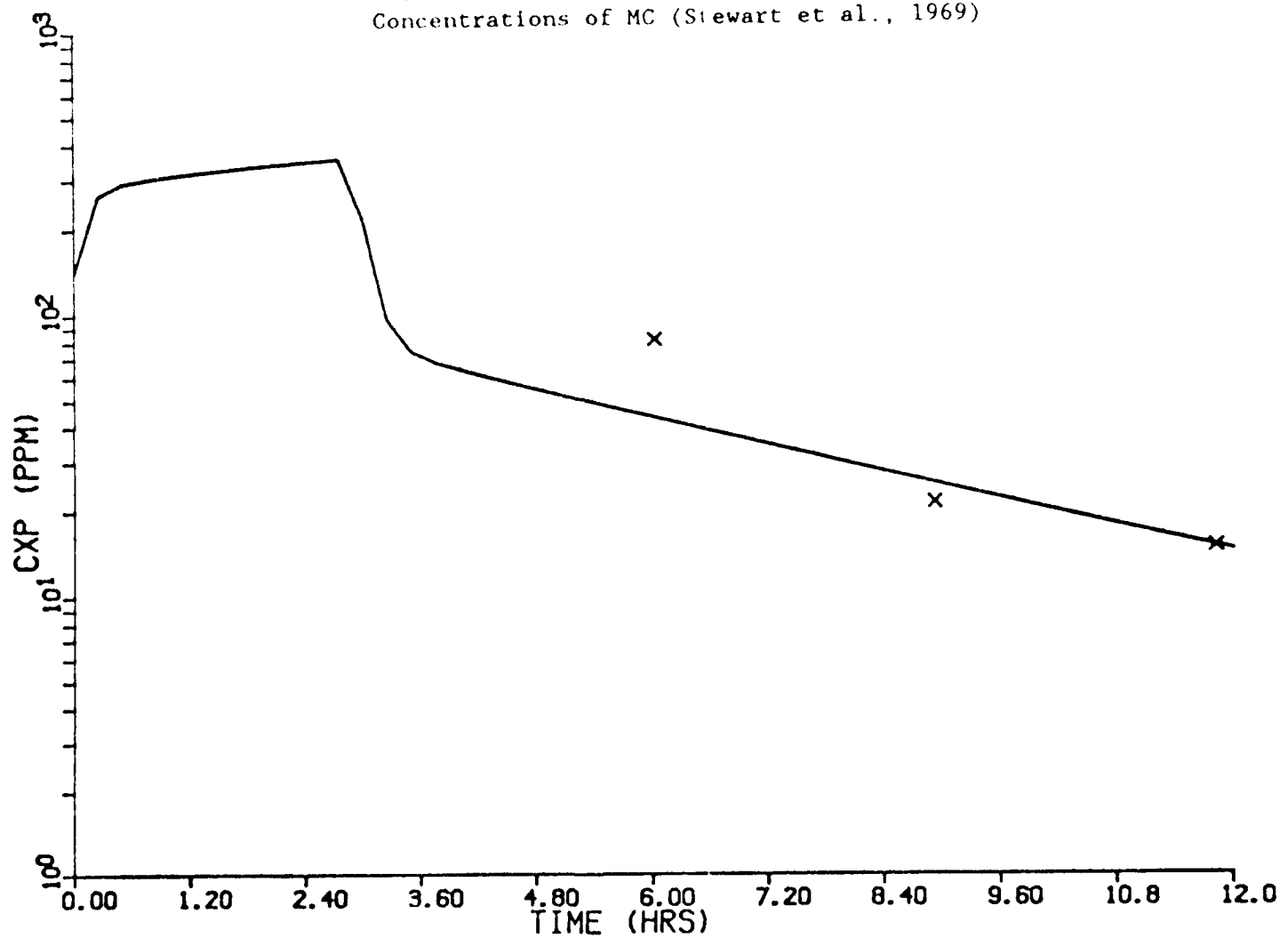
Observed and Predicted Alveolar Breath
Concentrations of MC (Stewart et al., 1969)



Observed concentrations from Stewart et al. (1969) for a 7-hour, 482 ppm exposure. Solid lines represent model predictions and x's represent observations.

Figure IV-9b

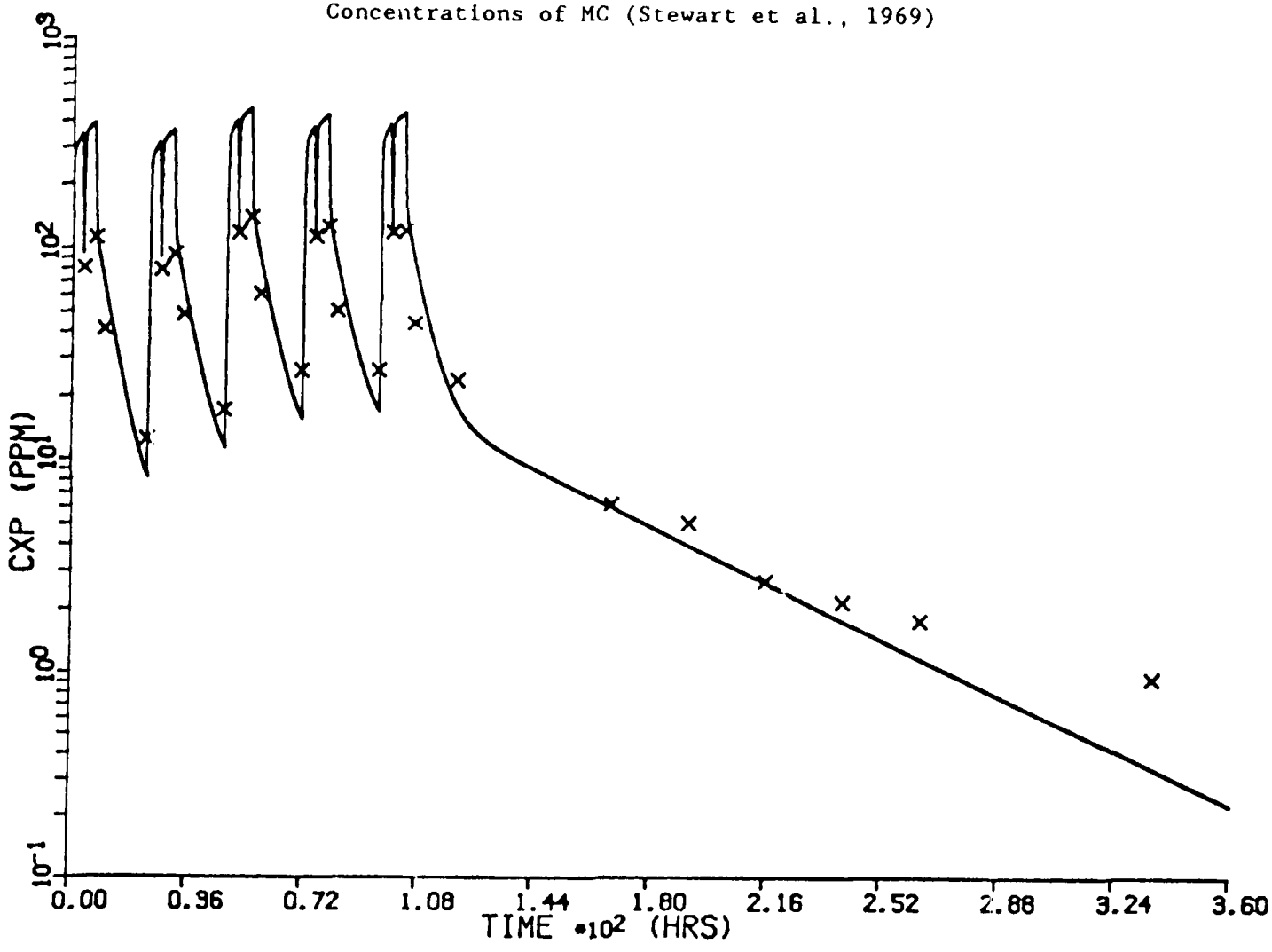
Observed and Predicted Alveolar Breath Concentrations of MC (Stewart et al., 1969)



Observed concentrations from Stewart et al. (1969) for a 3-hour, 507 ppm exposure. Solid lines represent model predictions and x's represent observations.

Figure IV-9c

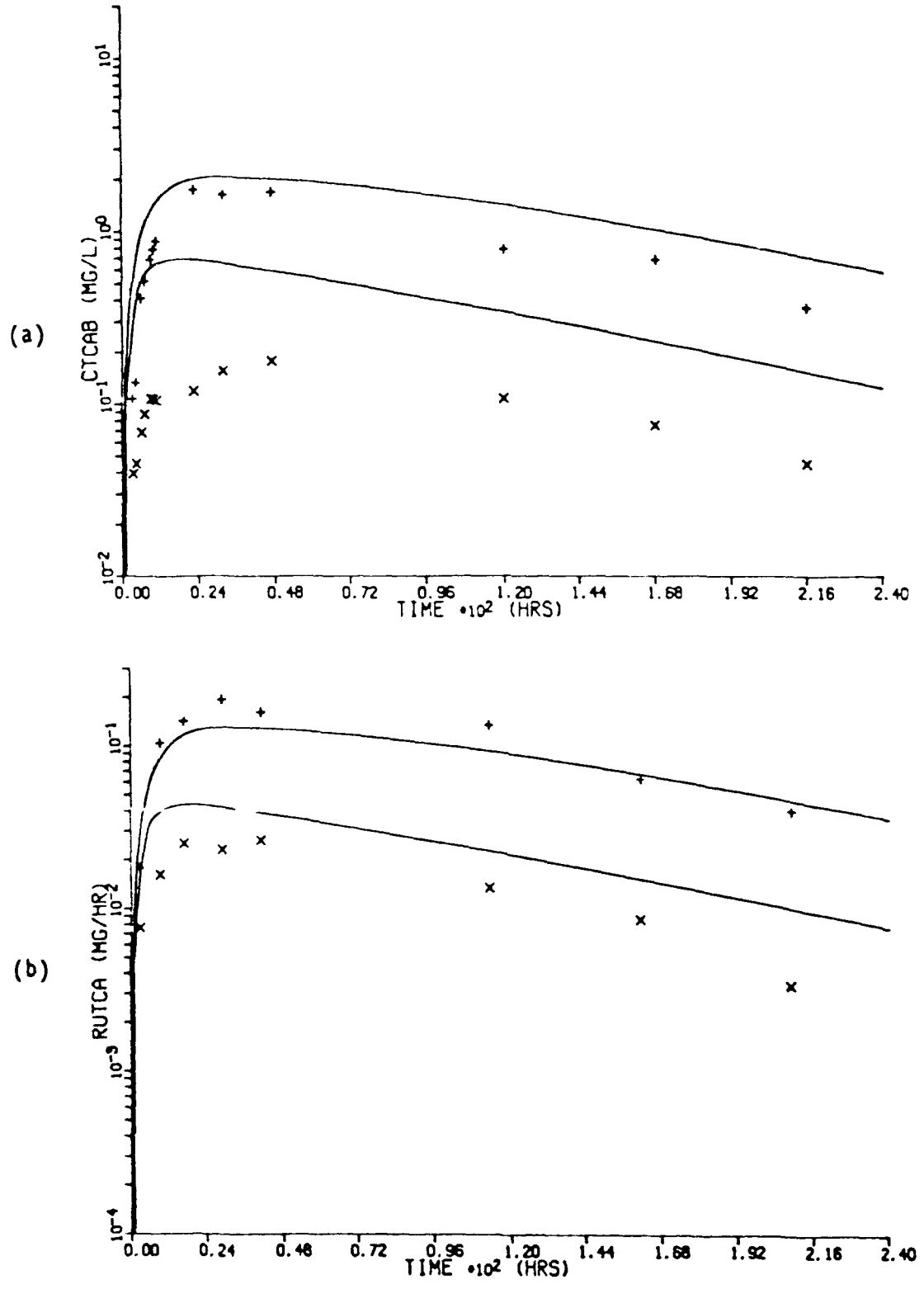
Observed and Predicted Alveolar Breath Concentrations of MC (Stewart et al., 1969)



Observed concentrations from Stewart et al. (1969) for five, 7- to 7.5-hour exposures averaging 507 ppm. Solid lines represent model predictions and x's represent observations.

Figure IV-10

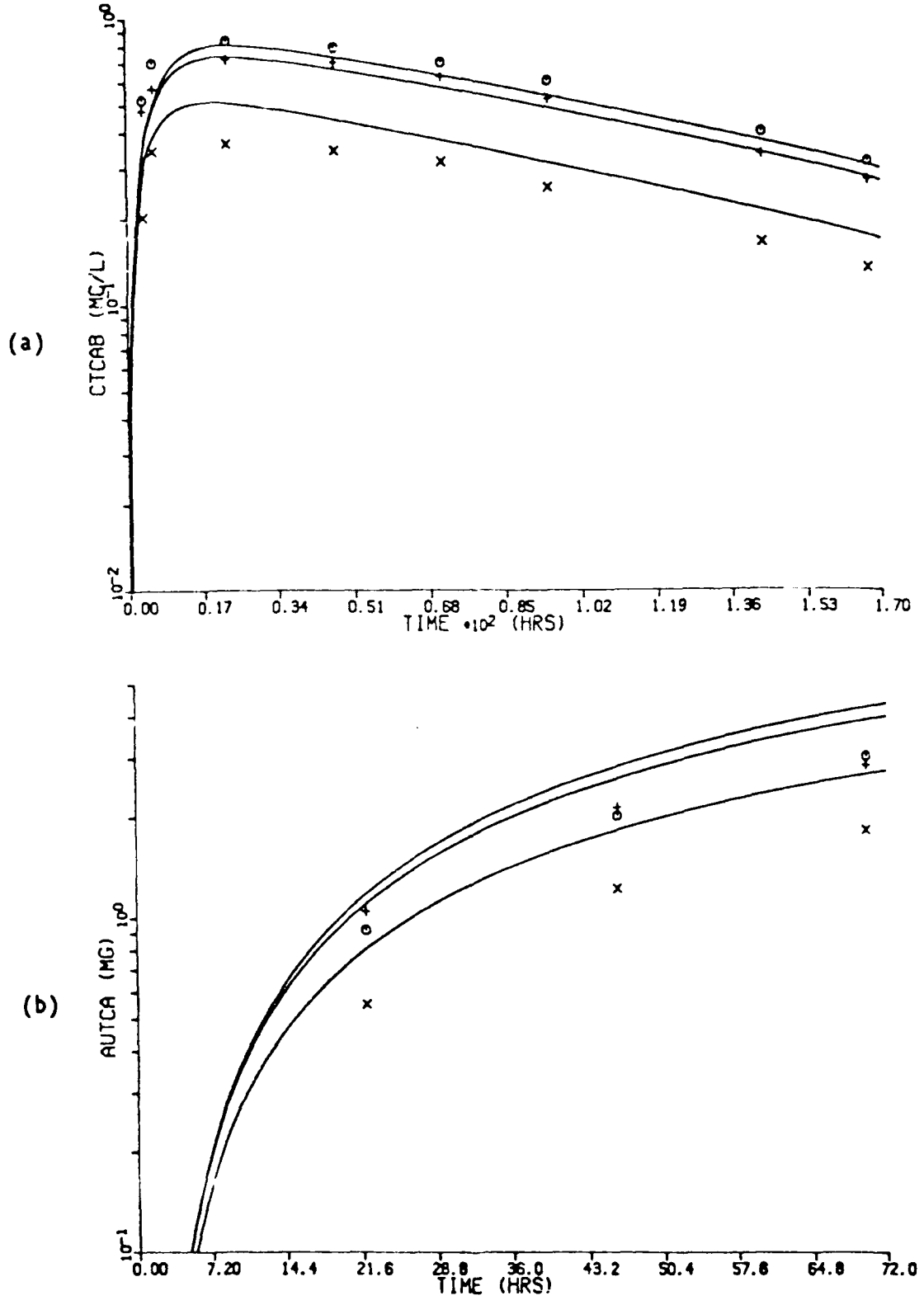
Observed and Predicted TCA Blood Concentrations and Urinary Excretion Rate (Nolan et al., 1984)



Observations from Nolan et al. (1984) for (a) TCA blood concentrations and (b) TCA urinary excretion rates during and following six-hour exposures to MC at concentrations of 35 ppm (x) and 350 ppm (+). Solid lines represent model predictions.

Figure IV-11

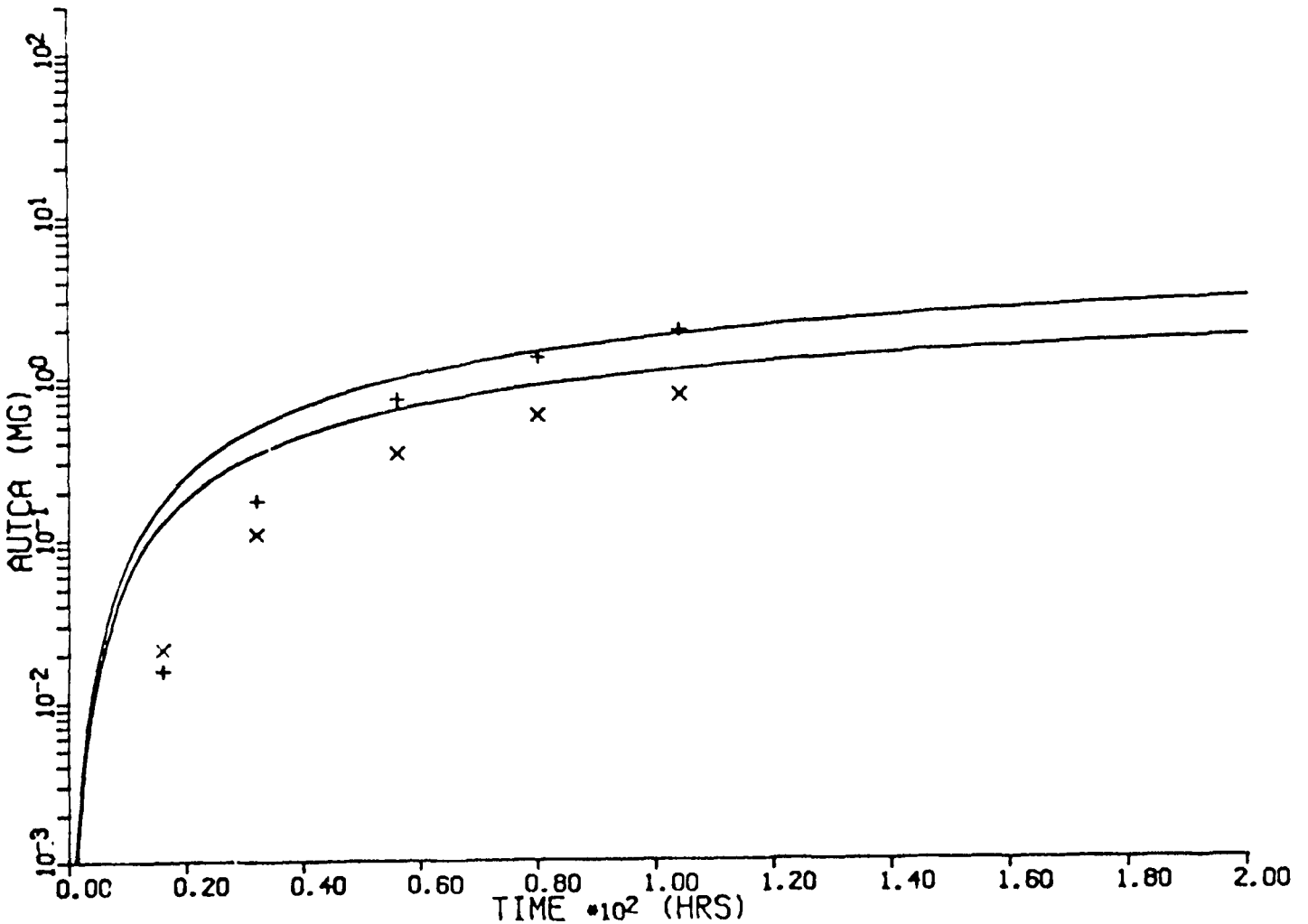
Observed and Predicted TCA Blood Concentrations
and Cumulative Amount Excreted (Monster et al., 1979)



Observations from Monster et al. (1979) for (a) TCA blood concentrations, and (b) cumulative amount of TCA excreted in urine during and following four-hour exposures to 72 ppm at rest (x), 144 ppm at rest (+), or 142 ppm with exercise alternating with rest (○). Solid lines represent model predictions.

Figure IV-12

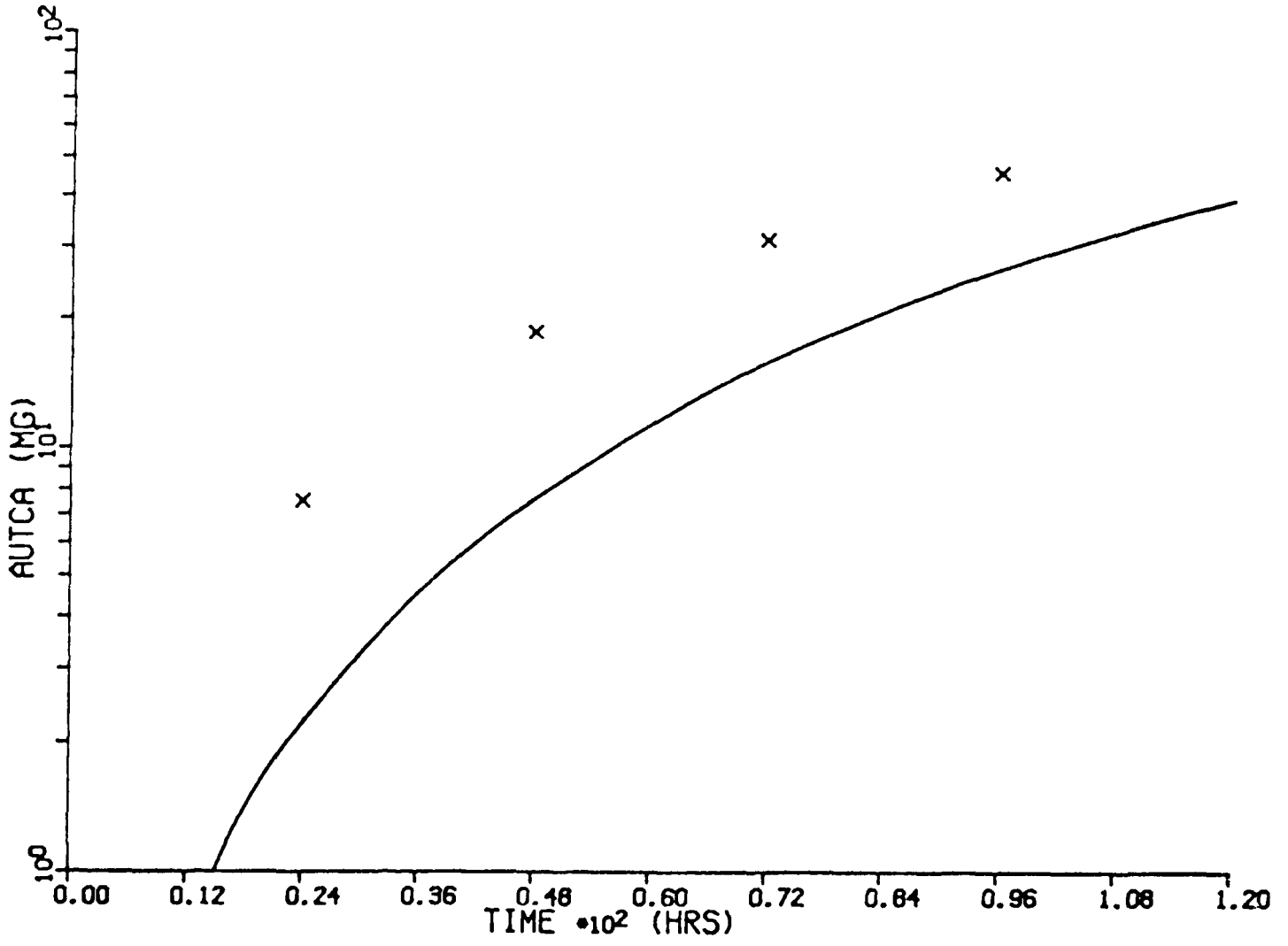
Observed and Predicted Cumulative TCA Excretion (Caperos et al., 1982)



Observed cumulative amount of TCA excreted in urine following eight-hour exposures to MC at concentrations of 72 ppm (x) or 213 ppm (+). Solid lines represent model predictions.

Figure IV-13

Observed and Predicted Cumulative TCA Excretion (Stewart et al., 1969)



Cumulative amounts of TCA excreted in urine during repeated 7- to 7.5-hour exposure to MC at concentrations averaging 507 ppm. Solid lines represent model predictions and x's represent observations.

APPENDIX VI-A

METHYL CHLOROFORM:
OVERVIEW OF TOXICITY AND PHARMACOKINETIC INFORMATION

APPENDIX VI-A

METHYL CHLOROFORM:
OVERVIEW OF TOXICITY AND PHARMACOKINETIC INFORMATION

Methyl chloroform (MC) has been tested for toxicity in mice, rats, rabbits, guinea pigs, monkeys, and dogs (Adams et al., 1950; Torkelson et al., 1958; McNutt et al., 1975; Prendergast et al., 1967; Klaassen and Plaa, 1967; Gehring, 1968; Eben and Kimmerle, 1974; Quast et al., 1988). The majority of these studies exposed animals via inhalation, which is the most probable route of exposure for humans. Relatively minor liver injury has been observed in mice, rats, dogs, and guinea pigs following exposure to high concentrations of MC (Adams et al., 1950; McNutt et al., 1975; Quast et al., 1988; Klaassen and Plaa, 1967). Reversible biochemical changes have been observed in dogs following exposure to high concentrations of MC; these parameters returned to normal 7 to 10 days post-exposure (Klaassen and Plaa, 1967). Necrosis or fatty degeneration of the liver has been observed in mice (McNutt et al., 1975) and guinea pigs (Adams et al., 1950) following exposure to MC. However, these two species may be more susceptible to toxicity because of a greater percentage uptake of MC (Torkelson et al., 1958). This greater percentage of uptake results in more MC available for metabolism to the toxicant.

Other evidence suggests that MC causes no liver effects in animals following exposure to high concentrations (Prendergast et al., 1967; Eben and Kimmerle, 1974). In many of the studies in which liver effects were reported, the concentrations needed to result in liver damage caused strong anesthetic effects and death in many of the animals. Therefore, certain investigtors have claimed that one need not be concerned about MC's effect on the liver (Gehring, 1968; Adams et al., 1950).

Several bioassays conducted to test the carcinogenicity of MC via inhalation and oral exposure have been reported (Quast et al., 1988, 1978; NCI, 1977a; NTP, 1983; Weisburger, 1977). However, many of these bioassays have been discredited due to treatment for less than lifetime of the animals (Quast et al., 1978), poor survival of the animals (NCI, 1977a), or data discrepancies (NTP, 1983). Of the remaining bioassays (Quast et al., 1988; Weisburger, 1977), liver tumors have been observed in mice following oral and inhalation exposures, but were not statistically significantly increased compared to untreated controls.

The pharmacokinetics of MC have been studied in rats, mice, and humans (Dallas et al., 1989; Ikeda and Ohtsuji, 1972; Schumann et al., 1982a,b; Nolan et al., 1984; Monster, 1979; Eben and Kimmerle, 1974; Caperos et al., 1982). The kinetics of MC depend upon its partition coefficients and species-specific physiology (Dallas et al., 1989) and appear to be very similar for all routes of exposure (Reitz et al., 1988).

The greater the cardiac output and pulmonary flow of an animal, the greater the percentage uptake of MC via inhalation (Dallas et al., 1989). For example, mice have a greater cardiac output and pulmonary flow per kilogram of body weight than those of rats, and cardiac output and pulmonary flow in rats is greater than those in humans. Therefore, it appears that, on a body weight basis, mice would absorb more MC than rats and have more MC available for metabolism. The greater the amount metabolized, the greater the toxicity of MC. This theory is confirmed by most of the results in the toxicity literature (McNutt et al., 1975).

The blood/air partition coefficient plays a large part in how much MC is absorbed. The blood/air partition coefficient for MC has been reported to be

1.6 to 5 (Nolan et al., 1984; Monster, 1979; Sato and Nakajima, 1979). MC rapidly equilibrates with alveolar blood because of this low blood/air partition coefficient. The percent uptake of MC in humans has been reported as high as 95% upon initiation of a 4-hour exposure; by the end of the exposure period, uptake had decreased to 30% (Monster et al., 1979).

MC has been reported to have a high fat/blood partition coefficient of approximately 108 (Nolan et al., 1984; Sato and Nakajima, 1979). Although this would tend to make MC concentrate in adipose tissue, actual distribution to adipose tissue occurs only to a minor extent because of the rapid release of solvent from blood to air. That is, very little MC is available for deposition in adipose tissue (Monster, 1979).

The majority of MC that is absorbed by rats, mice, and humans following inhalation exposure is excreted unchanged in the expired air (87% to 98%) within 53 hours post-exposure (Schumann et al., 1982a; Nolan et al., 1984). It has been reported that a small percentage of the MC that remains in the body is excreted unchanged in the urine (Imbriani et al., 1988). However, some of the MC that remains in the body is metabolized by the liver to trichloroethanol (Monster, 1979). Trichloroethanol can then be further metabolized to trichloroacetic acid (Monster, 1979), which may be the ultimate carcinogen.

APPENDIX IV-B

EQUATIONS DEFINING THE MC/TCA PBPK MODEL

APPENDIX IV-B

EQUATIONS DEFINING THE MC/TCA PBPK MODEL

MC

Gas Exchange Compartment

$$CA = (QC*CV + QP*CI)/(QC + QP/PB)$$

Fat Compartment

$$dCF/dt = QF*(CA-CVF)/VF$$

Rapidly Perfused Tissue Compartment

$$dCR/dt = QR*(CA-CVR)/VR$$

Slowly Perfused Tissue Compartment

$$dCS/dt = QS(CA-CVS)/VS$$

Liver Compartment

$$dCL/dt = QL*(CA\ CVL)/VL - dCL1/dt - dCL2/dt + (DRINK+GAV)/VL$$

$$dCL1/dt = Vmax*CVL/(VL*(Km+CVL))$$

$$dCL2/dt = Kf*CVL$$

Mixed Venous Blood

$$CV = (QL*CVL + QF*CVF + QR*CVR + QS*CVS)/QC$$

TCA

$$dCTCA/dt = PO*(dCL1/dt)*VL*(MWTCA/MWMC)/Vd - Ke*CTCA$$

$$dUTCA/dt = PU*Ke*CTCA*Vd$$

$$CTCA_B = 0.6*CTCA$$

Ci - Concentration of MC in i

i - F for fat
R for rapidly perfused tissues
S for slowly perfused tissues
L for liver perfused tissues
A for arterial blood leaving gas exchange compartment
V for mixed venous blood
I for inhaled air

CVi - Concentration of MC in venous blood leaving compartment i (i=L, F, R, S); CVi=Ci/pi

CL1 - Virtual concentration of MC metabolized via MFO pathway

CL2 - Virtual concentration of MC metabolized via first-order pathway

DRINK - Rate of MC introduction into liver compartment via drinking water

GAV - Rate of MC introduction into liver compartment via gavage

Vmax - $Vmaxc*bw^{0.7}$

Kf - $Kfc*bw^{-.3}$

CTCA - Concentration of TCA in plasma

UTCA - Cumulative amount of TCA eliminated in urine

$CTCA_B$ - Concentration of TCA in blood

MWTCA - Molecular weight of TCA

MWMC - Molecular weight of TCE

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